2.6 NONCLINICAL SUMMARY

SECTION 2.6.4—PHARMACOKINETICS WRITTEN SUMMARY

EMTRICITABINE/RILPIVIRINE/TENOFOVIR ALAFENAMIDE FIXED-DOSE COMBINATION (FTC/RPV/TAF FDC)

Gilead Sciences

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CONFIDENTIAL AND PROPRIETARY INFORMATION

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LIST OF ABBREVIATIONS

A-B	apical to basal
ADME	absorption, distribution, metabolism, and excretion
AhR	aryl hydrocarbon receptor (AHR gene product)
ATV	atazanavir (Reyataz®, Bristol-Myers Squibb)
B-A	basal to apical
BCRP	breast cancer resistance protein (ABCG2)
BDC	bile duct-cannulated
BID	twice daily
BNPP	bis-p-nitrophenyl phosphate
BSEP	bile salt export pump
CA	citric acid
Caco-2	human colonic adenocarcinoma cell line
CatA	cathepsin A
CEM-R1	human T-cell leukemic lymphoblast cells line
CES1	carboxylesterase 1
cDNA	complementary deoxyribose nucleic acid
CHO	Chinese hamster ovary cell line
CNS	central nervous system
COBI	cobicistat (GS-9350), Tybost®, Gilead
CsA	cyclosporine A
CSF	cerebrospinal fluid
CYP	cytochrome P450
DMSO	dimethyl sulfoxide
DRE	drug/dioxin response element
E/C/F/TAF	elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (coformulated)
EDTA	ethylenediamine tetra-acetic acid
EVG	elvitegravir (GS-9137; JTK-303 [Vitekta®, Gilead])
EVG/COBI/FTC/TDF	elvitegravir/cobicistat/ emtricitabine/tenofovir disoproxil fumarate (coformulated) (Stribild®, Gilead)
EU	European Union
FDC	fixed-dose combination
FMO	flavin-containing monooxygenase
FTC	emtricitabine (Emtriva®, Gilead)
f_u	fraction unbound
GD	gestation day
GI	gastrointestinal
GLP	good laboratory practice
GS-7340	tenofovir alafenamide (TAF) free base
GS-7340-02	tenofovir alafenamide (TAF) monofumarate

LIST OF ABBREVIATIONS (CONTINUED)

GS-7340-03	tenofovir alafenamide (TAF) hemifumarate
HIV, HIV-1	human immunodeficiency virus, type 1
HLM	human liver microsomes
HPLC	high performance liquid chromatography
HPMC	hydroxypropyl methylcellulose
IC_{50}	concentration required to produce 50% inhibition
LLOQ	lower limit of quantification
LSC	liquid scintillation counting
MATE1	multidrug and toxin extrusion protein 1 (SLC47A1)
MATE2-K	multidrug and toxin extrusion protein 2 (SLC47A2)
MDCK II	Madin-Darby canine kidney cell line
MDR1	P-glycoprotein (P-gp, ABCB1 gene product)
mRNA	messenger ribonucleic acid
MRP1	multidrug resistance-associated protein-1 (ABCC1)
MRP2	multidrug resistance-associated protein-2 (ABCC2, cMOAT)
MRP4	multidrug resistance-associated protein-4 (ABCC4)
MS/MS	Tandem mass spectrometry
MTT	3-[4,5-Dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (assay)
NADPH	β-nicotinamide adenine dinucleotide phosphate (reduced form)
NC	not calculated
ND	not detectable / not determined
MNR	nuclear magnetic resonance
NNRTI	nonnucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
NtRTI	nucleotide reverse transcriptase inhibitor
OAT1	organic anion transporter 1 (SLC22A6)
OAT3	organic anion transporter 3 (SLC22A8)
OATP	organic anion transporting polypeptide (SLCO or SLC22A gene products)
OATP1B1	organic anion transporting polypeptide 1B1 (SLCO1B1)
OATP1B3	organic anion transporting polypeptide 1B3 (SLCO1B3)
OCT2	organic cation transporter 2 (SLC22A2)
P_{app}	apparent permeability
PBMC	peripheral blood mononuclear cell
PEG400	polyethylene glycol 400
P-gp	P-glycoprotein (also see MDR1)
PI	protease inhibitor
PXR	pregnane X receptor (SXR, NR1I2 gene product)
QC	quality control
QWBA	quantitative whole-body autoradiography

LIST OF ABBREVIATIONS (CONTINUED)

RBC	red blood cell
RFD	radio flow-through detector
RLG	radioluminography
RPV, TMC278	rilpivirine (27.5 mg rilpivirine hydrochloride is equivalent to 25 mg rilpivirine)
RTV	ritonavir (Norvir®, Abbott)
S9	postmitochondrial (9,000 x g) supernatant
SD	standard deviation
STB	elvitegravir/cobicistat/ emtricitabine/tenofovir disoproxil fumarate fixed-dose single-tablet regimen (Stribild)
TDF	tenofovir disoproxil fumarate
TFV	tenofovir (GS-1278, R-PMPA)
UDPGA	uridine diphosphoglucuronic acid
UGT, UDP-GT	uridine diphosphate glucuronosyl transferase
US	United States
UV	ultraviolet

PHARMACOKINETIC ABBREVIATIONS

AUC	area under the plasma concentration versus time curve
$AUC_{0-\infty}$, AUC_{inf}	area under the plasma concentration versus time curve extrapolated to infinite time, calculated as $AUC_{0-last} + (C_{last}/\lambda_z)$
AUC_{x-xx}	partial area under the plasma concentration versus time curve from time “x” to time “xx” (default units are hours)
CL	systemic clearance of the drug after intravenous administration
CL/F	apparent clearance after oral administration of the drug
Cl_b	Total blood clearance
Cl_p	Total plasma clearance
C_{last}	last observed quantifiable concentration of the drug in plasma
C_{max}	maximum observed concentration of drug in plasma
C_x	plasma concentration at time "x" (default units are hours)
F	estimated oral bioavailability of the drug (%)
F_{abs}	Absolute oral bioavailability
λ_z	elimination rate determined from the terminal phase of the plasma concentration versus time curve
MAT	mean absorption time
MRT	mean residence time
$t_{½}$	half-life
t_{last}	time (observed time point) of C_{last}
t_{max}	time (observed time point) of C_{max}
V_{ss}	apparent steady-state volume of distribution of the drug
V_{ss}/F	apparent steady-state volume of distribution of the drug after oral administration

NOTE TO REVIEWER

This document contains a summary of nonclinical pharmacokinetic studies conducted in support of a fixed-dose combination (FDC) that contains the active substances emtricitabine (FTC, F), rilpivirine (RPV), and tenofovir alafenamide (TAF). The FDC tablet is referred to as emtricitabine/rilpivirine/tenofovir alafenamide (FTC/RPV/TAF) throughout this document.

As TAF is a new chemical entity, this nonclinical summary contains all available data on this new component while providing key data on FTC and RPV that support the efficacy and safety claims in the FDC prescribing information. Nonclinical studies have utilized TAF in most cases; however, relevant studies with the parent drug tenofovir (TFV) are also provided as applicable. Links to all study reports included in the dossier are highlighted in blue text.

In order to simplify the review, the order of presentation in each section follows the general format: FTC, RPV, and TAF (and TFV), followed by a summary of pharmacokinetic considerations for the FTC/RPV/TAF FDC. Given the lack of remarkable effects in the nonclinical studies with the individual agents, no additional pharmacokinetic studies have been conducted for the FTC/RPV/TAF combination.

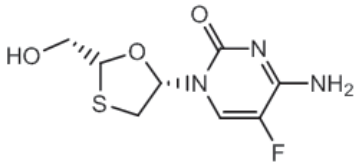
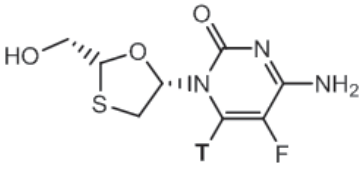
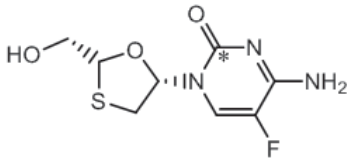
FTC

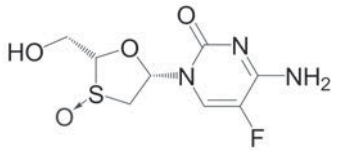
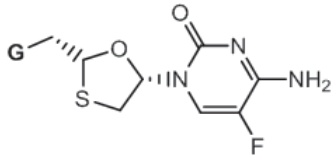
Structures of FTC and related compounds are illustrated in Table 1. The following conversions are also provided to aid the reviewer:

1 µM FTC (GS-9019) = 0.247 µg/mL

1 ng/mL FTC = 4.05 nM

Table 1. Names and Structures of FTC and Related Compounds

Name	Alternative Names	Identity	Structure
FTC	Emtricitabine, GS-9019, TP-0006, 524W91, Emtriva, Coviracil	Parent Compound	
[^{3}H]FTC	[6-^{3}H]FTC	Radiolabeled parent	
[^{14}C]FTC	[2-^{14}C]FTC	Radiolabeled parent	

Name	Alternative Names	Identity	Structure
M1, M2	M950, 3742W92, 3743W92	3'-Sulfoxide metabolites (2 diastereomers)	
M3		2'-O-Glucuronide metabolite	

*: Position of ^{14}C label
T: Tritium (^{3}H)
G: Glucuronic acid

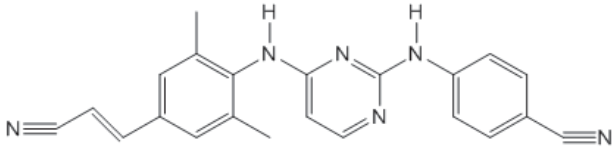
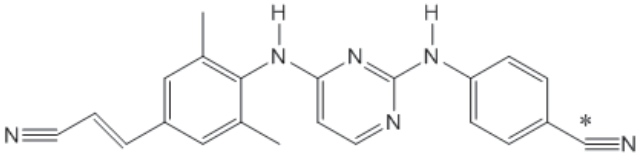
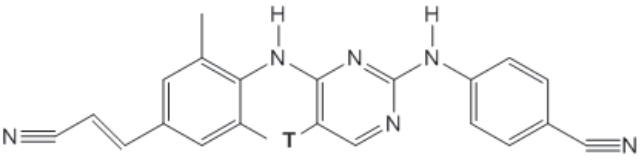
RPV

Structures of RPV and related compounds are illustrated in Table 3. The following conversions are also provided to aid the reviewer:

1 µM RPV (TMC278) = 0.366 µg/mL

1 ng/mL RPV = 2.73 nM

Table 2. Names and Structures of RPV and Related Compounds

Name	Alternative Names	Identity	Structure
RPV	Rilpivirine, TMC278	Parent Compound	
[^{14}C]RPV		Radiolabeled parent	
[^{3}H]RPV		Radiolabeled parent	

*: Position of ^{14}C label
T: Tritium (^{3}H)

TAF

Structures of TFV, TAF, and related compounds are illustrated in Table 3. The following conversions are also provided to aid the reviewer:

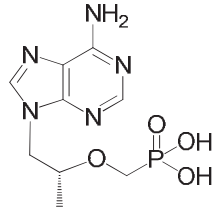
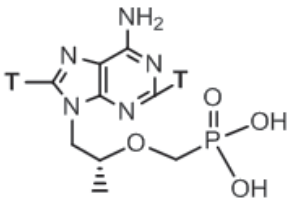
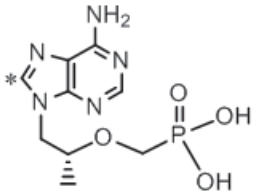
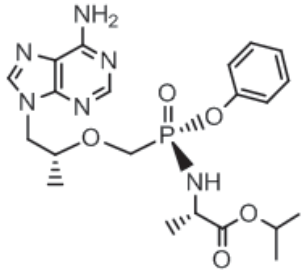
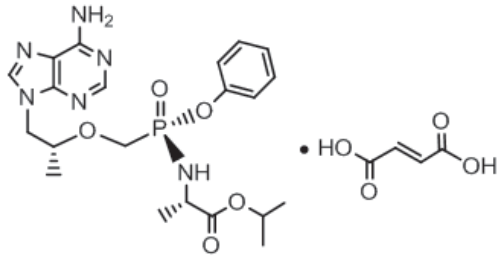
1 µM TFV (GS-1278) = 0.287 µg/mL

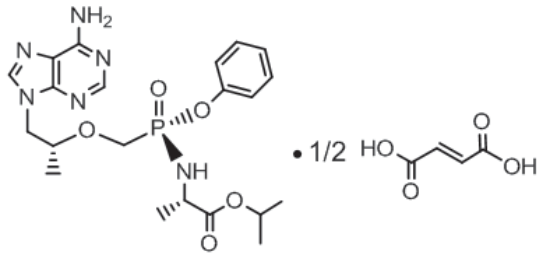
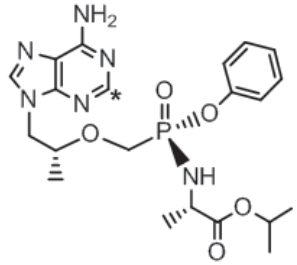
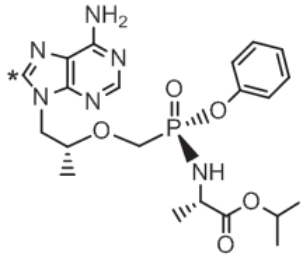
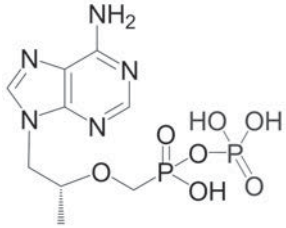
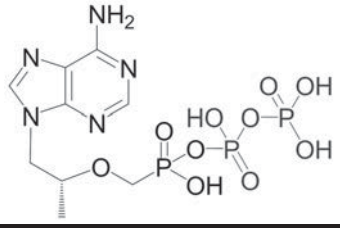
1 ng/mL TFV = 3.48 nM

1 µM TAF (GS-7340) = 0.477 µg/mL

1 ng/mL TAF = 2.10 nM

Table 3. Names and Structures of TFV, TAF, and Related Compounds

Name	Alternative Names	Identity	Structure
TFV	Tenofovir, GS-1278, R-PMPA	Parent Compound	
[^{3}H]TFV	[Adenine-2,8-^{3}H]TFV	Radiolabeled Parent	
[^{14}C]TFV	[Adenine-8-^{14}C]TFV	Radiolabeled Parent	
TAF (free base)		Parent Compound, free base	
GS-7340-02		Parent Compound, monofumarate	

Name	Alternative Names	Identity	Structure
GS-7340-03		Parent Compound, hemifumarate	
[^{14}C]TAF	[Adenine-2-^{14}C]TAF	Radiolabeled Parent	
	[Adenine-8-^{14}C]TAF	Radiolabeled Parent	
GS-342031	GS-77389, PMPAp, TFV-MP	Monophosphorylated Anabolite of Parent	
GS-077635	PMPApp, TFV-DP	Diphosphorylated Anabolite of Parent	

*: Position of ^{14}C label
T: Tritium (^{3}H)

1. BRIEF SUMMARY

This dossier is being submitted in support of a marketing application for a fixed-dose combination (FDC) that contains emtricitabine (FTC, F [Emtriva®]), rilpivirine (RPV, R [Edurant®], also referred to as TMC278 throughout this document) and tenofovir alafenamide (TAF, formerly GS-7340) for the treatment of human immunodeficiency virus-1 (HIV-1) infection: the FTC/RPV/TAF FDC (200/25/25 mg).

Comprehensive studies have been completed characterizing the absorption, distribution, metabolism, and excretion profiles of FTC, RPV, and TAF (and/or tenofovir [TFV]/tenofovir disoproxil fumarate [TDF]). In addition, the potential of each agent to be involved in pharmacokinetic drug-drug interactions has been characterized. This application contains all available data on each component. In order to simplify review, the order of presentation in each section follows the general format: FTC, RPV, and TAF studies, followed by a summary of pharmacokinetic considerations for the FTC/RPV/TAF FDC.

The nonclinical data discussed within this document support the proposed use of the F/R/TAF FDC for the treatment of HIV-1 infection. All information from nonclinical pharmacokinetic studies that is of relevance to the prescriber and patient has been included in the proposed prescribing information.

FTC

Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI), developed by Gilead, that is marketed as a once-daily capsule (200 mg). Emtricitabine is a synthetic analogue of the naturally occurring pyrimidine nucleoside, 2′-deoxycytidine, which is structurally similar to lamivudine.

Nonclinical studies to characterize the absorption and disposition of FTC have been performed in mice, rats, and primates. The pharmacokinetic studies are listed in the overview table (m2.6.5, Section 1), and study details are provided in the individual study overview tables in m2.6.5. The tabulated summaries in m2.6.5 also provide toxicokinetic data for studies described in m2.6.6.

In mice, rats, and cynomolgus monkeys, FTC was rapidly and extensively absorbed with oral bioavailability ranging from 58% to 97%. In general, there were no differences in pharmacokinetic profiles following single and multiple dosing. Systemic exposure to FTC (C_{max} and AUC) increased approximately proportionally with dose and was similar between males and females. With chronic dosing, somewhat higher exposures were observed in the mouse and rat studies when compared to short term dosing; however, there was no evidence of accumulation in the monkey studies.

Emtricitabine is widely distributed throughout the body, with a volume of distribution similar to that of total body water. After oral administration, the highest concentrations of FTC were found in the kidneys, intestine, and liver and exceeded those in plasma, while concentrations in CNS tissues were less than 10% of those in plasma. Emtricitabine was also readily transferred across the placenta. Emtricitabine is almost completely eliminated within 72 hours following dosing, with no evidence of tissue accumulation. Emtricitabine does not undergo extensive first-pass or

systemic metabolism, and is eliminated primarily by renal excretion of unchanged drug. The total body clearance of FTC exceeds the glomerular filtration rate, suggesting that the drug is actively secreted by the kidney.

Metabolism is a minor route of elimination and is similar in humans and monkeys. It includes oxidation of the thiol moiety (Phase 1 metabolism) to form the 3'-sulfoxide diastereomers (M1 and M2) and conjugation with glucuronic acid (Phase 2 metabolism) to form the 2'-O-glucuronide (M3). The most abundant metabolite was one of the 3'-sulfoxides (M1 or M2). Several minor metabolites account for < 2% of the dose and are eliminated primarily in the urine. Importantly, FTC is not converted to 不純物FA* . Oxidation of FTC is largely catalyzed by CYP3A, but flavin-containing monooxygenase (FMO) enzymes may also play a role. Emtricitabine does not inhibit human CYP and demonstrates no liability to be an inducer.

RPV

Rilpivirine (TMC278) is a nonnucleoside reverse transcriptase inhibitor (NNRTI) that is marketed as a once-daily tablet (Edurant® 25 mg) and as part of the RPV/FTC/TDF FDC (Complera®/Eviplera®) for the treatment of HIV-1 infection.

TMC278 has been examined in both in vitro and in vivo test systems. In vivo studies were conducted in CD-1 and CB6F1-nonTgrasH2-transgenic mice; pigmented Long Evans and Sprague Dawley rats; New Zealand white rabbits; beagle dogs; and cynomolgus monkeys. With the exception of pigmented rats, all species and strains were the same as those used in the nonclinical pharmacology and toxicology studies. Results from a mass-balance trial evaluating the metabolism and excretion of TMC278 in humans, as well as exposure to TMC278 in patients, are included to provide comparison with results obtained from studies in animal species and to support overall conclusions about the exposure, metabolism, and elimination of TMC278.

The pharmacokinetic studies are listed in the overview table (m2.6.5, Section 1), and study details are given in the individual study overview tables in m2.6.5. The drug substance, TMC278, has been used in 2 forms in the nonclinical studies, in base form and as the hydrochloride (HCl) salt form. The HCl salt form was used in a limited number of preclinical studies and in the clinical tablet formulations. Throughout this document, these forms are referred to as “TMC278 base” and “TMC278,” respectively. The dose levels used in all studies are expressed as base equivalent, and the appropriate correction factor was used when the HCl salt was administered. The form determined in the in vitro media or in in vivo compartments is referred to as “TMC278.”

Studies in human colon carcinoma-derived (Caco-2) cells revealed that TMC278 can be classified as a compound with an intermediate permeability. Passive transcellular diffusion was proposed as a mechanism for TMC278 absorption. However, the solubility, and in the case of suspension and solid dosage forms, possibly also dissolution seem to limit the rate and extent of absorption. After oral administration of TMC278 base, the absolute oral bioavailability of TMC278 was 32%, 54%, 31%, and 24% in rats, rabbits, dogs, and monkeys, respectively.

After oral gavage administration of TMC278 (2 forms), peak plasma concentrations were generally reached rapidly, followed by a decline at lower dose levels whereas at higher dose levels, the plasma profiles showed a plateau until at least 8 hours in all species. Across the dose range studied, plasma concentrations of TMC278 increased dose-proportionally but more often less than dose-proportionally due to poor solubility. At very high dose levels, no further increase in exposure was seen. There were no major gender differences in pharmacokinetics in dogs and in mice at low dose levels whereas in rats and in mice at high dose levels, exposures in females were higher than in males (up to 2-fold in mice and 4-fold in rats). In mice, exposure after repeated administration of TMC278 was comparable or slightly lower than that at Day 1 in males and females (only at 20 mg/kg/day). In females, at higher dose levels (60 to 320 mg/kg/day) exposure after repeated administration was higher (up to 2.2-fold) than at Day 1. In rats, after repeated administration of the base, systemic exposure increased slightly (up to 1.6-fold) in comparison to Day 1 in females, while in males particularly at high dose levels, exposure decreased slightly (up to 40%). After repeated administration of the HCl salt, the decrease in exposure seen in males was more pronounced (up to 76%) compared to base, particularly in the carcinogenicity study, while no further decrease was observed between Week 27 and Week 39. In female rats no clear tendency for time-dependent pharmacokinetics was seen. In rabbits, the exposures obtained after repeated administration were similar to those obtained at Day 1. In dogs, after repeated administration, the exposure increased compared to Day 1 mainly due to the long half-life ($t_{1/2}$ = 31 hours) of TMC278. In monkeys, after repeated administration of TMC278, exposure had a tendency to increase but due to the high inter-individual variability it is difficult to reach firm conclusions.

In rats, tissue distribution of [^{14}C]TMC278 and its metabolites after a single dose was rapid and extensive. The highest concentrations of radioactivity were measured in the liver, adrenal gland, brown fat, and kidney. There was no evidence of undue retention, and there were no indications of irreversible binding of TMC278 and its metabolites to melanin. In pregnant rats, there was distribution of [^{14}C]-TMC278 to the placenta and the fetus. Total radioactivity exposure values in the placenta and in whole fetus were 0.94- and 0.64-fold those of maternal blood, respectively.

TMC278 is extensively bound to plasma proteins and this is independent of the concentration and species. In the various animal species and human, plasma protein binding ranged from 99.08% to 99.97%. TMC278 is extensively bound to human albumin and to a much lesser extent to α_1-acid glycoprotein. The distribution of TMC278 to red blood cells is limited in all species.

Some differences were seen in clearance across species. In rats, blood clearance of TMC278 is moderate, whereas in rabbits, dogs, and monkeys it is low compared to the hepatic blood flow. Large differences in $t_{1/2}$ of TMC278 were observed between the rat (4.4 hours), rabbit (12 hours), dog (31 hours), and monkey (7.1 hours). The volume of distribution at steady-state was larger in rats, dogs, and monkeys, and very low in rabbits.

TMC278 is metabolized by Phase I and Phase II pathways including aromatic and aliphatic hydroxylation, glutathione conjugation, N-glucuronidation, and nitrile split-off followed by reduction/oxidation; whether or not in combination with secondary pathways such as glucuronidation, dehydration, and catabolism of the glutathione conjugate. In mice, oxidation of TMC278, and to a lesser extent glutathione conjugation were the predominant pathways. In rats,

the glutathione conjugation pathway was the predominant pathway, whereas in dog and man, oxidation of TMC278 was the predominant one. No unique human metabolites were observed. In plasma of animals and human, unchanged TMC278 was more abundant than any metabolite.

In rodents dosed with [^{14}C]TMC278, total radioactivity was rapidly excreted, whereas in dogs, excretion was relatively slow. In all animal species and human, the predominant route of excretion was via feces (> 85%) and generally, the majority of the total radioactivity eliminated was unchanged TMC278. Renal excretion of total radioactivity was very limited (0.45% to 6.1% of the dose) in all animal species and human and the amount of unchanged TMC278 in urine was negligible. In rats, biliary excretion was limited (18% to 25% of the dose) and the amount of unchanged TMC278 in bile was negligible. In rats, there was an indication that TMC278 was excreted in milk.

In vitro, the cytochrome P450 (CYP450) 3A4 isoenzyme plays a major role in the biotransformation of TMC278. TMC278 might be a very weak inducer of CYP1A2 and CYP2B6 and a moderate inducer of CYP2C19 and CYP3A4. TMC278 is an inhibitor of CYP2C8 and CYP2C9 in vitro, whereas no inhibition is expected in vivo. TMC278 was shown to have P-glycoprotein (P-gp, MDR1) inhibitor properties with an apparent inhibitory concentration of 50% (IC_{50}) value of 9.2 µM (3.4 µg/mL). Inhibition of transepithelial permeation of P-gp substrates cannot be excluded, but any effect is unlikely to be clinically relevant. P-glycoprotein inhibitors are not expected to play a role in modulating the intestinal absorption of TMC278 given the limited role of efflux transporters in the epithelial permeability of TMC278.

Ex vivo induction studies in rodents showed that TMC278 is an inducer of the CYP3A-family (up to 1.7-fold in mice and up to 6-fold in rats) and CYP4A-family (up to 25-fold in mice and up to 4.7-fold in rats). Additionally, TMC278 induced uridine diphosphate-glucuronosyltransferase (UDP-GT) activity in mice (up to 2.3-fold) and to a lesser extent in rats (up to 1.3-fold only at high dose in males). In dogs, treatment with TMC278 did not result in any enzyme induction.

The recommended dose of TMC278 in HIV-infected treatment-naive patients is 25 mg once daily. At this dose level, mean C_{max} (Weeks 4–8) was 0.13 µg/mL and mean $AUC_{0–24h}$ (Week 48; population PK) was 2.4 µg·h/mL (m2.7.2, Sections 3.2.1.4.2 and 3.2.1.3.2, [TMC278-TiDP6-C209 and TMC278-TiDP6-C215]). These values were compared with those obtained at the highest doses tested in animal species.

At the end of administration, the C_{max} and AUC ratios (animal/human) ranged from 1.9 to 2.4 in monkeys, 25 to 42 in dogs, 97 to 115 in pregnant rabbits, 7.5 to 48 in male rats, 35 to 123 in female rats, 63 to 100 in pregnant rats, 21 to 70 in juvenile rats, and 210 to 446 in mice.

TAF and TFV

Tenofovir alafenamide is a prodrug of TFV, a nucleotide reverse transcriptase inhibitor (NtRTI). Tenofovir alafenamide is more stable in plasma than TDF, allowing for it to circulate for a longer period of time and interact with target cells. Tenofovir alafenamide provides enhanced delivery of TFV to the liver and HIV-target cells and tissues, resulting in higher intracellular levels of the active phosphorylated metabolite TFV-diphosphate (TFV-DP). In lymphoid cells and tissues, TAF is predominantly hydrolyzed intracellularly to TFV by cathepsin A (CatA) {13119}. Once released intracellularly, TFV is metabolized to the active metabolite, TFV-DP {1574}, a competitive inhibitor of HIV-1 reverse transcriptase that terminates the elongation of

the viral DNA chain {21}, {1131}. The increased stability of TAF in plasma combined with the enhaced delivery of TFV to the target cells and tissues results in higher intracellular levels of TFV-DP and lower circulating levels of TFV relative to TDF {17137}.

Nonclinical studies to characterize the absorption and disposition of TAF have been performed in mice, rats, dogs, and monkeys. The pharmacokinetic studies are listed in the overview table (m2.6.5, Section 1), and study details are provided in the individual study overview tables in m2.6.5. The tabulated summaries in m2.6.5 also provide toxicokinetic data for studies described in m2.6.6. For the pharmacokinetic studies using GS-7304-02 or GS-7340-03, all doses are presented as TAF free base equivalent.

Tenofovir alafenamide was rapidly absorbed with t_{max} values of 0.08 hours in mouse and dog and 0.5 hours in monkey (the first time point assessed). Tenofovir alafenamide was undetectable in any of the rat plasma samples. Thereafter, TAF plasma concentrations declined rapidly with a terminal half-life of less than 1 hour. Concomitant with the rapid decline in TAF concentrations in plasma, the predominant metabolite TFV was formed and persisted in plasma over the dosing interval.

Protein binding of TAF was moderate in human plasma with the percent unbound of 46.8% in vitro which was higher than values observed in multiple human ex vivo studies where the mean percent unbound of TAF ranged from 14% to 23% in all subjects. Following oral administration of [^{14}C] TAF to mouse, rat, and dog, [^{14}C]TAF-derived radioactivity was widely distributed to most of the tissues in all species studied. Consistent with high hepatic extraction, high levels of radioactivity were observed in the liver. High levels of radioactivity were also measured in the kidney. Low levels of radioactivity were observed in brain and testis in mouse. No evidence for melanin binding was observed in rats. Distribution trends in the pigmented uveal tract of the eye and pigmented skin suggested that [^{14}C]TAF-related radioactivity was not selectively associated with melanin-containing tissues in the pigmented mouse.

Metabolite profiling of TAF in mice, rats, dogs and humans demonstrated formation of purine metabolites that are also present endogenously including hypoxanthine, xanthine, allantoin, and uric acid in all species including humans. Tenofovir accounted for a majority of drug-related material in plasma, urine, and feces from all species except for human plasma in which uric acid was the predominant metabolite accounting for 73.9% of the total AUC over 96 hours. No metabolites unique to human were observed. Tenofovir alafenamide is not an inhibitor of UGT1A1 and CYP enzymes except for weak inhibition observed for CYP3A in vitro. While TAF is a weak inhibitor of CYP3A in vitro, it is not a clinically meaningful inhibitor of CYP3A. Tenofovir alafenamide is not a clinically relevant inducer of CYP enzymes, UGT1A1, or P-gp. Tenofovir alafenamide was not an inhibitor of any of the transporters tested in vitro indicating that TAF is unlikely to be a perpetrator of transporter-mediated drug interactions. Tenofovir alafenamide was found to be a substrate for efflux transporters P-gp and BCRP and hepatic uptake transporters OATP1B1 and OATP1B3. A modest increase in TAF absorption has been observed upon inhibition of the intestinal efflux transporters in vitro and in vivo. Exposure to TAF may be affected by inhibitors of OATP1B1 and OATP1B3 or by genetic polymorphisms affecting their transport activities. The effects of differences in OATP1B1 and OATP1B3 activity are, however, not expected to be clinically relevant given the high passive permeability of TAF.

Following oral dosing of mice, rats, and dogs with [^{14}C]TAF, the majority of radiolabel is recovered in the feces or urine in all species. The elimination of a large amount of radioactivity in bile of bile duct-cannulated (BDC) dogs indicates that biliary excretion is a major route of elimination of [^{14}C]TAF-derived radioactivity in dogs. Total recovery of radiolabel was high for all species. Renal excretion was identified as the primary route of elimination of TFV in all species tested, and is achieved by a combination of glomerular filtration and active tubular secretion. In vitro transport studies indicate that the active tubular secretion of TFV in humans is mediated by OAT1 and MRP4 acting in series, as the major uptake and efflux transporters in proximal tubules, respectively. Human OAT3 may play a secondary role in the tubular uptake of TFV. Neither P-gp nor MRP1 or MRP2 are involved in the tubular efflux of TFV. As the primary transporter for the tubular uptake of TFV, OAT1 has been assessed for its potential as a target for drug-drug interactions between TFV and other renally secreted therapeutics including antibiotics, anti-inflammatory agents, and other antivirals (including COBI and PIs). Under physiologically relevant conditions, a number of renally excreted drugs showed no effect in vitro on the OAT1-mediated transport of TFV. Similarly, PIs and COBI did not exhibit any effect on the cellular elimination of TFV mediated by the MRP4 efflux pump in vitro, indicating that PIs and COBI are unlikely to exert any substantial effect on the accumulation of TFV in renal proximal tubules or renal elimination of TFV. Tenofovir did not inhibit the activity of the renal uptake transporter, OCT2, or the renal efflux transporter, MATE1.

FTC/RPV/TAF

Based upon the differences in routes of elimination, RPV is unlikely to affect the pharmacokinetic profiles of FTC or TFV adversely. The effect of P-gp inhibition by RPV observed in vitro is not clinically relevant as little or no change in exposures of the P-gp substrates, digoxin or TAF, was observed when codosed with RPV. Pharmacokinetic interactions between FTC and TAF are not anticipated as FTC, TAF, and TFV are not inhibitors or inducers of drug metabolizing enzymes and are not inhibitors of any of the transporters tested. In addition, FTC and TAF are analogs of 2 different nucleosides, cytidine and adenosine, respectively, and do not share a common intracellular metabolism pathway for pharmacological activation through phosphorylation. Neither TAF nor FTC is expected to affect the pharmacokinetic profiles of RPV since they are not clinically meaningful inducer or inhibitor of drug metabolizing enzymes and transporters.

2. METHODS OF ANALYSIS

2.1. FTC

The in vivo pharmacokinetic, toxicokinetics, distribution, and excretion of FTC were assessed in mouse, rat, and monkey. The in vitro metabolism and drug interaction characteristics of FTC were studied in appropriate model systems.

2.1.1. Bioanalytical Methods Supporting Toxicokinetic Studies

Analytical methods used to quantify FTC in mouse, rat, and monkey plasma from the early preclinical absorption, distribution, metabolism, and excretion studies employed reverse-phase high performance liquid chromatography (HPLC) with ultraviolet detection at 280 nm. The LLOQ was 0.063 to 0.125 µg/mL {4251}. Additional mouse, rat, rabbit, and monkey pharmacokinetic and toxicokinetic studies used HPLC-MS-based assays for the quantitation of FTC in plasma and urine. Initially, a method employing selected ion monitoring was developed (m2.6.5, Section 2.1, 97/001.01) and this was subsequently improved by incorporation of MS/MS detection (m2.6.5, Section 2.1, 6447 v5, 7582 v1, and 6159 v1). Methods were cross-validated, and the LLOQ was generally in the range of 0.100 to 0.200 µg/mL.

2.1.2. Other In Vivo Methods

The recovery of radioactivity in urine and feces was determined after administration of [^{3}H]FTC to CD-1 mice (m2.6.5, Section 10.1.1, TEIN/93/0015) and samples were also subject to LC-radioprofiling. The recovery of radioactivity in feces and urine after administration of [^{14}C]FTC to Sprague-Dawley and Long Evans rats was determined (m2.6.5, Section 5.1.1, TOX092). Samples were subject to LC-radioprofiling and tissue distribution was determined by QWBA. Radioactive recovery and LC-radioprofiling studies were performed with cynomolgus monkeys after administration of [^{3}H]FTC (m2.6.5, Section 10.1.3, TEIN/93/0016) or [^{14}C]FTC (m2.6.5, Section 10.1.2, TOX063).

2.1.3. In Vitro Methods

The extent of binding of [^{3}H]FTC in plasma from mouse, rabbit, monkey, and human plasma was determined by equilibrium dialysis (m2.6.5, Section 6.1.1, TBZZ/93/0025). The potential for FTC to inhibit human CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A was determined by examining its effects on selective activities catalyzed by human hepatic microsomal fraction (m2.6.5, Section 13.1.1, 15247). Positive control inhibitors were tested in parallel. The effect of FTC on glucuronidation by human hepatic microsomal fraction was also determined using 7-hydroxycoumarin as a general UGT substrate. The potential for FTC to be a substrate for human CYP enzymes was determined with 9 individual bacterially expressed CYP enzymes and through the use of human hepatic microsomal fraction and enzyme-selective inhibitors (m2.6.5, Section 8.1.1, 15396V1). Positive control substrates were tested in parallel. The role of glucuronide conjugation in the metabolism of FTC was also determined with human hepatic microsomal fraction and UDPGA as the cofactor.

2.2. RPV

2.2.1. Radio-Labeled TMC278

A large number of studies discussed in this summary were conducted with radiolabeled TMC278. Two radiolabeled (^{14}C and ^{3}H) TMC278 compounds were used but most of the studies were performed with ^{14}C. The ^{14}C atom was on the nitrile carbon of the benzonitrile of the TMC278 molecule (Table 2). The original material had a radiochemical purity of 98.2% and a specific activity of 2.03 GBq/mmol. The ^{3}H atom was in the pyrimidine moiety of TMC278 molecule. The radiochemical purity was 99.7% and the specific activity was 8.07 TBq/mmol (Table 2), and was used in one study to investigate plasma protein binding and distribution in blood.

The metabolic stability of the ^{14}C label of TMC278 was investigated following a single oral dose of [^{14}C]TMC278 at 10 mg/kg in Sprague Dawley rats (TMC-FK4686). The recovery of [^{14}C]O_2 from the expired air collected for 25 hours after administration was negligible. This indicates that the ^{14}C label is metabolically stable.

2.2.2. Radiochemical Methods

The detailed description of radiochemical methods used in ADME studies is given in the various study reports. The following techniques were used:

- Tissue distribution of total radioactivity was studied by quantitative whole-body autoradiography (QWBA) in male pigmented rats and pregnant female Sprague Dawley rats (see Section 4.2.2.1). The concentration of radioactivity in the different tissues was determined by radioluminography, whereas the concentration of radioactivity in the eye and in biological fluids (blood and plasma) was determined by liquid scintillation counting (LSC).

- Total radioactivity in biological samples was measured by LSC, using appropriate scintillation cocktails. Aliquots of biological fluids were counted directly (plasma, urine, and bile) following extraction or combustion (blood and feces residues).

In metabolism and/or mass balance studies, unchanged compound and/or its major metabolites were determined in various biological samples (plasma, urine, bile, and feces). Mass balance was based on the recovery of radioactivity from various samples or pools of samples. In in vitro and in vivo studies with [^{14}C]TMC278, metabolite profiles were determined by radio-HPLC. Metabolite identification was done by a combination of liquid chromatography with tandem mass spectrometry (LC-MS/MS) and co-chromatography with synthesized metabolites (see Sections 5.2.2 and 5.3.2).

2.2.3. Bioanalytical Methods

Bioanalytical methods were developed to support the TMC278 toxicokinetic and pharmacokinetic program. Methods were all based on the same detection technique, i.e., tandem mass spectrometry. The performance of most of these assays was characterized by validation

processes, in line with the internal procedures and bioanalytical guidelines {15698}, {15551}, (Bioanalytical Method Validation, Guidance for Industry. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Veterinary Medicine May 2001). The validation data for each assay are described in detail in the respective method validation reports.

An LC-MS/MS method was validated for the determination of TMC278 in mouse (m2.6.5, Section 2.2, TMC278-FK4240), rat (m2.6.5, Section 2.2, TMC278-FK4170), rabbit (m2.6.5, Section 2.2, TMC278-BA1014), dog (m2.6.5, Section 2.2, TMC278-FK4169), and monkey (m2.6.5, Section 2.2, TMC278-NC273) ethylenediamine tetra-acetic acid (EDTA) plasma, and dog (m2.6.5, Section 2.2, TMC278-FK4169) heparin plasma. Tissue samples were analyzed with non-validated qualified research methods based on the validated plasma methods. The validation data for LC-MS/MS methods (heparin and EDTA plasma) are summarized below and details are outlined in the respective method validation reports.

In-study validation was conducted for nonclinical GLP studies. These validation data are appended to the individual preclinical study reports.

2.2.4. LC-MS/MS Methods

The LC-MS/MS method for mouse, rat, rabbit, and dog plasma consisted of a solid-phase extraction (SPE) followed by reversed-phase HPLC coupled to tandem mass spectrometry.

The selectivity of the LC-MS/MS assay towards endogenous compounds was proven in 6 different batches of non-pooled blank EDTA or heparin plasma.

The inter-batch accuracy and inter-batch precision were calculated by comparing the theoretical concentration with the mean measured concentration for sets of QC samples at 4 concentrations (LLOQ, Low, Medium, and High). The accuracy was within the criteria of 80% to 120% at the LLOQ QC level and 85% to 115% at the other levels. The inter-batch precision of the LLOQ QC was ≤ 20% and ≤ 15% at the other levels. Detailed information on the accuracy and precision can be found in the method validation reports.

2.2.5. Stability

The stability of TMC278 was assessed in the stock solution solvent (methanol) and in biological matrices (both heparin and EDTA blood and plasma at several temperatures). The test article was found not to be stable in daylight. When TMC278 is exposed to daylight, the drug is transformed to the Z-isomeric form. Therefore, each assay was carried out under yellow-light conditions and samples were protected from light.

TMC278 was stable in methanol for at least 6 months after storage in a freezer (–18°C), for 1 month in a refrigerator (4°C ± 2°C), and for 3 days at room temperature (yellow-light conditions) (m2.6.5, Section 2.2, TMC278-FK4170).

2.3. TAF

The in vivo pharmacokinetic, toxicokinetics, distribution, and excretion of TAF were assessed in mouse, rat, dog, and monkey. The in vitro absorption, metabolism, and drug interaction characteristics of TAF were studied in appropriate model systems.

2.3.1. Bioanalytical Methods Supporting Pharmacokinetic Studies

In the early preclinical absorption studies TFV levels in rat plasma and TAF and TFV levels in dog plasma and peripheral blood mononuclear cells (PBMCs) were determined using a fluorescence derivitization/HPLC procedure (m2.6.5, Section 3.3, R990130, 99-DDM-1278-PK). Analyses of TAF and TFV in plasma and PBMCs during pharmacokinetic studies following single or multiple oral administration to rats (m2.6.5, Section 3.3, R2000065, AD-120-2015), mice (m2.6.5, Section 3.3, AD-120-2014, AD-120-2016; m2.6.7, Section 6.3, TX-120-2006), dogs (m2.6.5, Section 3.3.8, AD-120-2034 and m2.6.5, Section 14.3.15, AD-120-2035), and monkeys (m2.6.5, Section 3.3.9, P2000087) were performed using LC/MS/MS (m2.6.5, Section 2.3, BA-120-2003, BA-120-2004, BA-120-2010, BA-120-2011, BA-120-2012, and BA-120-2013). Some of these methods did not strictly conform to Good Laboratory Practice (GLP) guidelines but were evaluated for appropriate selectivity, sensitivity, linearity, as well as intra-assay accuracy and precision.

2.3.2. Bioanalytical Methods Supporting GLP Studies

Validated methods of HPLC with MS detection were used for the earlier rat toxicokinetic studies (m2.6.5, Section 2.3, 001092/NGE and R-BA-Tox-120-001). Plasma concentrations of TAF and TFV were quantified in toxicology studies in pregnant mouse, rat, and rabbit using validated LC/MS/MS methods (m2.6.5, Section 2.3, BA-120-2003, BA-120-2004, and BA-120-2005). In a 28-day toxicology study in dog, the plasma TAF concentrations were determined using a validated LC/MS/MS assay with a limit of quantification of 5 ng/mL and the concentrations of TFV in plasma and in PBMCs were determined using fluorescence derivitization followed by HPLC with fluorescence detection (m2.6.5, Section 2.4, P1278-0017) and HPLC with MS detection (m2.6.5, Section 2.3, 993680 MYS), respectively. Analytical methods for determination of TAF in plasma and TFV in plasma and PBMCs from a 9-month dog toxicology study (m2.6.5, Section 2.3, TOX-120-002) are described within the appendices of this study report.

In a 28-day repeat dose study in monkeys, validated methods were used to determine TAF and TFV in plasma (m2.6.5, Section 2.3, 010520/PDW and 010521/PHZ) and TFV in PBMCs (m2.6.5, Section 2.3, AA01240-RQZ).

Two assay techniques were used to determine plasma TFV concentrations. The first was a reverse phase ion-pair HPLC method following fluorescence derivatization (m2.6.5, Section 2.4, P4331-00008 [mouse], P1278-00001 [rat, monkey], and P1278-00017 [dog]). This assay was validated with a LLOQ of 25 ng/mL, and was used in earlier studies. The second assay utilizes LC/MS/MS and was validated with LLOQs of 1–3 ng/mL (m2.6.5, Section 2.4, P1278-00028 [rat], P1278-00029 [monkey], and P4331-0037 [dog]). The LC/MS/MS assay was also validated for the determination of TFV in rat milk with an LLOQ of 10 ng/mL (m2.6.5, Section 2.4, P1278-00034).

2.3.3. Other In Vivo Bioanalytical Methods

The absorption, distribution, metabolism, and excretion of TAF were assessed in various species following a single oral administration of [^{14}C]TAF. The location of [^{14}C] label at the 2- or 8-position of the adenine base is indicated by an asterisk in the structures described in Table 3. The tissue distribution of [^{14}C]TAF-derived material was assessed using QWBA of pigmented and nonpigmented male mice and rats or by LSC in dogs (m2.6.5, Section 5.3, AD-120-2009, AD-120-2011, and AD-120-2020). The metabolism and excretion of [^{14}C]TAF has been assessed in intact male mice, and in intact and BDC rats and dogs (m2.6.5, Section 10.3, AD-120-2008, AD-120-2012, and AD-120-2021). HPLC or LC/MS/MS coupled with radio flow-through detector (RFD) was used for metabolite profiling and identification. Tenofovir alafenamide and its metabolites were separated using reverse phase chromatography, and detected using an RFD and MS simultaneously. The retention times of the metabolites using reverse phase chromatography were determined by the peaks on radiochromatograms generated by an inline RFD, and the molecular ions of the metabolites were determined on the full scan mass spectra by tandem MS corresponding to the retention times of metabolites on the radiochromatograms. Tandem MS of the molecular ions was performed, and the structures of the metabolites were proposed based on their mass spectra. Where possible, the structures of the metabolites were confirmed by comparison of the chromatographic and mass spectral characteristics of the metabolites with authentic reference standards.

2.3.4. In Vitro Methods

2.3.4.1. Permeability Across Caco-2 Cell Monolayers

In vitro bidirectional permeability of TAF (incubated at 10 µM) was assessed using monolayers of the human colonic adenocarcinoma cell line Caco-2, on 12 well transwell dual chamber plates. Permeability rates were determined by quantifying the TAF levels in each chamber by LC/MS/MS. Experiments were done in the absence or in the presence of COBI, a known inhibitor of P-gp (m2.6.5, Section 14.3.3, AD-120-2013).

2.3.4.2. Stability

The stability of TAF has been assessed in plasma, intestinal S9 and hepatic S9 fractions from dog and human (m2.6.5, Section 8.3, AD-120-2025, AD-120-2024, and AD-120-2023). Intestinal stability of TAF was also studied in the presence of HIV PIs or pharmacoenhancers using intestinal S9 fractions (m2.6.5, Section 14.3.5, AD-120-2027). The disappearance of the parent prodrug was monitored by LC/MS/MS.

The stability of [^{14}C]TFV in rat hepatic microsomal fractions and in liver S9, intestinal S9, and plasma from dog and human was determined by LC-radioprofiling (m2.6.5, Section 8.3.6, 96-DDM-1278-003).

CYP-mediated metabolism was assessed with CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 by incubating TAF at 5 µM with bacterially expressed each human CYP450 enzyme preparations (Bactosomes) coexpressed with human NADPH CYP reductase in the presence of NADPH (m2.6.5, Section 8.3.4, AD-120-2004). The disappearance of the parent prodrug was monitored by LC/MS/MS.

2.3.4.3. Intracellular Metabolism

Intracellular activation of TAF was assessed in primary human hepatocytes. Tenofovir alafenamide was continuously incubated with primary human hepatocytes for 24 hours and cell samples were collected at select time point followed by washing and extraction. The samples were analyzed by LC/MS/MS and intracellular concentrations of GS-77389 were quantified (m2.6.5, Section 8.3.5, AD-120-2017 and m2.6.5, Section 14.3.7, AD-120-2031).

2.3.4.4. Plasma Protein Binding

The binding of TAF to plasma was assessed by equilibrium dialysis against phosphate buffer at 37°C using pooled plasma from Beagle dogs and humans (m2.6.5, Section 6.3.1, AD-120-2026). Unbound TAF was quantified by LC/MS/MS.

The binding of [^{3}H]TFV in human plasma was determined by ultrafiltration (m2.6.5, Section 6.3.2, P0504-00039.1).

2.3.4.5. Inhibition of Cytochrome P450 Enzymes and UGT1A1

The inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A was assessed by incubating TAF with human liver microsomes and NADPH in the presence of individual probe substrates. Each probe substrate metabolite was quantified by LC/MS/MS. The following positive control inhibitors for each CYP isoform were used to establish the validity of the assay; α-naphthoflavone (CYP1A2), ticlopidine (CYP2B6), montelukast (CYP2C8), sulfaphenazole (CYP2C9), tranylcypromine (CYP2C19), quinidine (CYP2D6), and ketoconazole (CYP3A4) (m2.6.5, Section 13.3.1, AD-120-2003). The mechanism-based inhibition was assessed using CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A following a 2-step incubation protocol. First, TAF or positive control inhibitors were incubated with human liver microsomes in the presence or absence of NADPH for 30 min at 37°C. The mixture was then diluted 10-fold with a phosphate buffer containing the respective probe substrate and fresh NADPH to initiate the second incubation for the CYP reaction. Each probe substrate metabolite was quantified by LC/MS/MS. The following positive control inhibitors for each CYP isoform were used to establish the validity of the assay: resveratrol and furafylline (CYP1A2); ticlopidine (CYP2B6); gemfibrozil glucuronide (CYP2C8); tienilic acid (CYP2C9); ticlopidine (CYP2C19); paroxetine (CYP2D6); and mibefradil and mifepristone (CYP3A4) (m2.6.5, Section 13.3.3, AD-120-2040).The inhibition of UGT1A1 was assessed by incubating TAF (up to 50 μM) with insect cell microsomal fraction containing expressed human UGT1A1, UDP-glucuronic acid, and a probe substrate, estradiol at 37°C. Formation of the metabolite, estradiol 3-glucuronide was monitored by LC/MS/MS (m2.6.5, Section 13.3.6, AD-120-2006).

The effects of TFV on human CYP1A2, CYP2C9, CYP2E1, CYP2D6, and CYP3A activity were determined with human hepatic microsomal fraction and enzyme-selective activities (m2.6.5, Section 13.3.2, V990172-104).

2.3.4.6. Induction Potential

The potential of TAF to induce human drug metabolizing enzymes and transporters through the activation of AhR and PXR was assessed in vitro in reporter cell lines (m2.6.5, Section 13.3.4, AD-120-2005). Briefly, assessments of induction were done using Puracyp's hepatoma-derived cell lines, DRE12.6 and DPX2. DPX2 cells are stably transformed with an expression vector for human PXR and a reporter gene vector containing the enhancer regions of CYP3A4 linked to luciferase. DRE12.6 cells are transformed with an expression vector for human AhR and the drug/dioxin response element (DRE) of the human CYP1A2 gene linked to a luciferase reporter.

The induction potential of TAF on CYP activity and CYP, P-gp, and UGT1A1 mRNA levels was assessed in primary human hepatocytes (m2.6.5, Section 13.3.5, AD-120-2032). Three preparations of cryopreserved human hepatocytes isolated from 3 separate livers were treated once daily for 3 consecutive days with dimethyl sulfoxide (DMSO, 0.1% v/v, vehicle control), 1 of 3 concentrations of TAF (1, 10, or 100 μM). For positive controls for induction, omeprazole (CYP1A2) at 50 μM, phenobarbital (CYP2B6 and P-gp) at 100 μM, rifampin (CYP3A4) at 10 μM, or β-naphthoflavone (UGT1A1) at 20 μM were used. After treatment, the cells were incubated with CYP probe substrate for the analysis of phenacetin O dealkylation (marker for CYP1A2), bupropion hydroxylation (marker for CYP2B6), and testosterone 6β hydroxylation (marker for CYP3A4) by LC/MS/MS. After the activity assay, RNA was analyzed by qRT PCR to assess the effect of TAF on CYP1A2, CYP2B6, CYP3A4, P-gp, and UGT1A1 mRNA levels. In addition, a test to assess toxicity potential of TAF to human hepatocytes during the course of induction treatment was conducted with the same lots of cryopreserved human hepatocytes using the 3-[4,5-Dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.

2.3.4.7. Interaction with Transporters

The potential for TAF and TFV to be a substrate for human P-gp was assessed using monolayers of Caco-2 cells, or MDCK II cells transfected with an expression vector for the protein, and through the use of chemical inhibitors (m2.6.5, Section 14.3.1, AD-120-2018, m2.6.5, Section 14.3.9, AD-104-2002). The potential for TAF to inhibit P-gp or BCRP was assessed using MDCK II cells expressing each of the tansporters (m2.6.5, Section 14.3.2, AD-120-2019). The potential of TAF to be a substrate or an inhibitor of human OATP1B1 and OATP1B3 were evaluated using CHO cells (AD-120-2019; m2.6.5, Section 14.3.4, AD-120-2022). Interactions of TAF with human OAT1, OCT1, MATE1, and OCT2 were studied using CHO cells expressing the individual transporters (m2.6.5, Section 14.3.6, AD-120-2036). The effect of TAF on the human bile salt export pump (BSEP) and OAT3 was determined using the transporter expressing Sf9 cell membrane vesicles and Flp-In 293 cells, respectively (AD-120-2036).

Interactions of TFV with human MRP2 were evaluated with the human ovarian carcinoma cell line, 2008, transfected with an expression vector for the protein (m2.6.5, Section 14.3.8, AD-104-2001). Interactions of TFV with human MRP4 were performed with the human T-cell leukemic lymphoblast cells line, CEM-R1, overexpressing the protein, and were compared with the CEM-SS parental line. The potential for TFV to be a substrate for human OAT3, OCT1, or OCT2 was determined by examining uptake of [^{3}H]TFV by microinjected Xenopus laevis oocytes (m2.6.5, Section 14.3.14, PC-103-2001). The effect of TFV on human MRP1 activity

was determined using MDCK II cells overexpressing the protein (m2.6.5, Section 14.3.12, PC-104-2014). The effect of TFV on human OCT2 and MATE1 activities was determined using CHO cells expressing the individual proteins (m2.6.5, Section 14.3.13, AD-104-2012).

2.4. FTC and TFV

2.4.1. In Vitro Transporter Methods

The effects of FTC and TFV (the major metabolite of TAF) on P-gp and BCRP were tested using MDCKII cells (m2.6.5, Section 14.4, AD-236-2003, AD-236-2004, AD-236-2005). The potential for FTC and TFV to be inhibitors of OATP1B1 and OATP1B3 was tested in CHO cells expressing the individual transporters (m2.6.5, Section 14.4.5, AD-236-2006). The effects of FTC and TFV on OCT2 and MATE1 were assessed in CHO cells (m2.6.5, Section 14.4.1, AD-236-2001 and Section 14.4.9, AD-236-2011). The effects of FTC on OAT1 and OAT3 were tested in CHO cells and on MRP4 were evaluated using vesicles containing the transporter (m2.6.5, Section 14.4.6, AD-236-2007 and Section 14.4.8, AD-236-2010). The effects of FTC and TFV on OCT1 were examined in CHO cells and the effects on BSEP were tested using vesicles (m2.6.5, Section 14.4.7, AD-236-2008). The potential for FTC to be substrate or inhibitor of MRP2 was assessed using vesicles (m2.6.5, Section 14.4.10, AD-236-2012 and Section 14.4.11, AD-236-2013).

3. ABSORPTION

3.1. In Vitro Absorption Studies

3.1.1. FTC: In Vitro Absorption Studies

Emtricitabine shows high, dose-independent bioavailability in vivo in mice (Section 3.2.1.1) and monkeys (Section 3.2.1.2); thus, in vitro absorption studies were not considered necessary.

3.1.2. RPV: In Vitro Absorption Studies

Human colon carcinoma-derived Caco-2 cells were used as an in vitro model to investigate the transepithelial transport characteristics of [^{14}C]TMC278 in both the absorptive (apical to basolateral) and the secretory (basolateral to apical) directions at initial donor concentrations between 3 and 300 µM (1.1 and 111 µg/mL). In addition, in order to evaluate the role of P-gp in TMC278 transport, TMC278 at 30 µM (11 µg/mL) was incubated in the presence of the P-gp inhibitor verapamil (100 µM). Possible inhibition of P-gp mediated transport of taxol (75.8 nM) across Caco-2 cells was also investigated in the presence of increasing concentrations of TMC278 (1-100 µM; 0.37-37 µg/mL) (m2.6.5, Section 3.2.1, TMC278-NC104).

TMC278 showed apparent permeability coefficients ranging from 10×10^{-6} to 13×10^{-6} cm/s and 15×10^{-6} to 27×10^{-6} cm/s for absorptive and secretory transport, respectively. Comparison of these permeation rates with those of the reference compounds alniditan (low permeability), levocabastine (medium permeability), and theophylline (high permeability) indicated that TMC278 can be classified as a compound with an intermediate permeability.

The permeability of TMC278 across the monolayers was concentration-independent between 3 and 300 µM (1.1 and 111 µg/mL). TMC278 permeation at steady state across the monolayers was slightly polarized with efflux ratio (secretory/absorptive permeation) values of 1.5 to 2. Coincubation with the P-gp inhibitor verapamil did not affect TMC278 transport. These results indicate that TMC278 permeation occurs predominantly by passive transcellular diffusion and that TMC278 is not a substrate of P-gp.

Bidirectional transport experiments with the P-gp substrate taxol demonstrated that TMC278 has P-gp inhibitory properties with an apparent IC_{50} value of 9.2 µM (3.4 µg/mL).

Based on the above, it is concluded that TMC278 will exhibit sufficient membrane permeability to obtain adequate intestinal absorption, provided solubility and dissolution are not rate limiting. Inhibition of transepithelial permeability of P-gp substrates by TMC278 can not be excluded, but for most drugs a possible effect is unlikely to be clinically significant at the intestinal absorption level. TMC278 did not affect plasma exposure to P-gp substrates including digoxin and TAF in clinical drug interaction studies (GS-US-120-0117 and GS-US-120-1554).

The results of the present study are consistent with previously reported results using unlabeled TMC278 in Caco-2 monolayers (TMC278-TiDP6-JRF [FK4155]).

3.1.3. TAF: In Vitro Absorption Studies

Permeability of TAF was studied in both apical to basolateral (forward) and basolateral to apical (reverse) directions at 10, 100, and 1000 µM using Caco-2 monolayers (m2.6.5, Section 3.3.1, AD-120-2037). Tenofovir alafenamide showed a dose dependent increase in forward permeability and a decrease in efflux ratio indicating saturable efflux transport (Table 4). At 100 µM TAF, the apparent forward permeability rate was 0.63 x 10^{-6} cm/s and the efflux ratio was 13.6. Addition of the efflux transport inhibitor, cyclosporine A (CsA) diminished the efflux ratio and increased the forward permeability.

Table 4. Dose-Dependent Bidirectional Permeability of TAF in Caco-2 Cells

Compound	Inhibitor	Concentration (µM)	Mean P_{app} (x10^{-6} cm/s)		Efflux Ratio
			Forward	Reverse	
TAF	None	10	0.34	6.98	20.2
TAF	None	100	0.63	8.47	13.6
TAF	None	1000	1.08	5.86	6.28
TAF	10 µM CsA	10	1.51	1.34	1.00

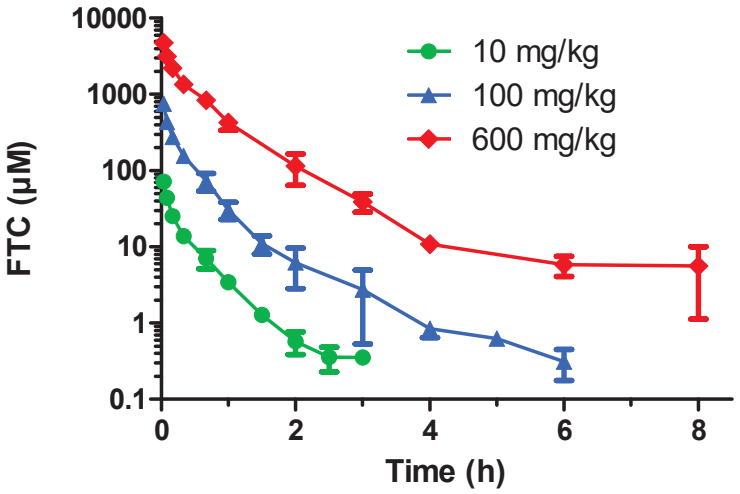
3.2. Single-Dose In Vivo Studies

3.2.1. FTC: Single-Dose In Vivo Studies

3.2.1.1. FTC: Mouse, Oral/Intravenous

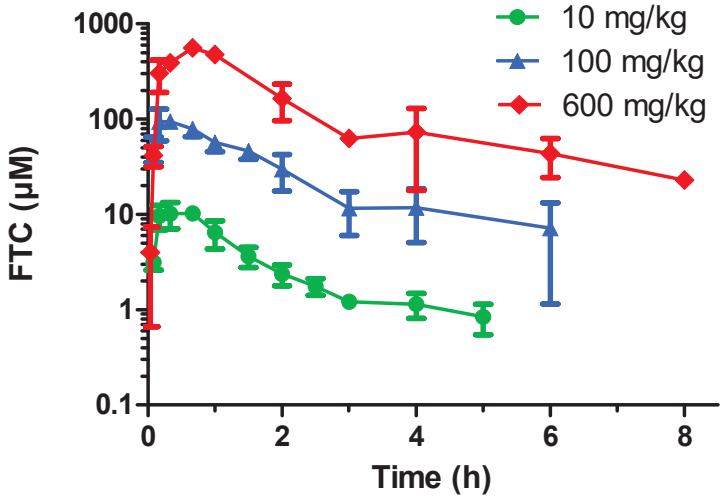
The FTC pharmacokinetic profile was determined after IV and oral administration to nonfasted male CD-1 mice at doses of 10 mg/kg (m2.6.5, Section 3.1.1, TEIN/93/0003), 100 mg/kg (m2.6.5, Section 3.1.2, TEIN/93/0004), and 600 mg/kg (m2.6.5, Section 3.1.3, [redacted]00101). Pharmacokinetic profiles are illustrated in Figure 1 and Figure 2. After IV administration, the decline in plasma concentration was bi- or tri-exponential, with V_{ss} values close to that of total body water (0.89–1.1 L/kg) and clearance values (1.28–2.33 L/h/kg) exceeding the glomerular filtration rate in mice. After oral administration, absorption was rapid and extensive, with absolute bioavailability values of 96%, 79%, and 63% at 10, 100, and 600 mg/kg, respectively. C_{max} and AUC values increased roughly dose-proportionally from 10 to 600 mg/kg.

Figure 1. **Mean Plasma Concentration vs. Time Profile Following an Intravenous Bolus Dose of FTC in Solution to Male CD-1 Mice (mean ± SD)**



FTC = emtricitabine; SD = standard deviation
Source: Reports TEIN/93/0003 (10 mg/kg, n=5), TEIN/93/0004 (100 mg/kg, n=5) and [redacted]00101 (100 mg/kg, n=3)

Figure 2. **Mean Plasma Concentration vs. Time Profile Following an Oral Dose of FTC in Solution to Male CD-1 Mice (mean ± SD)**



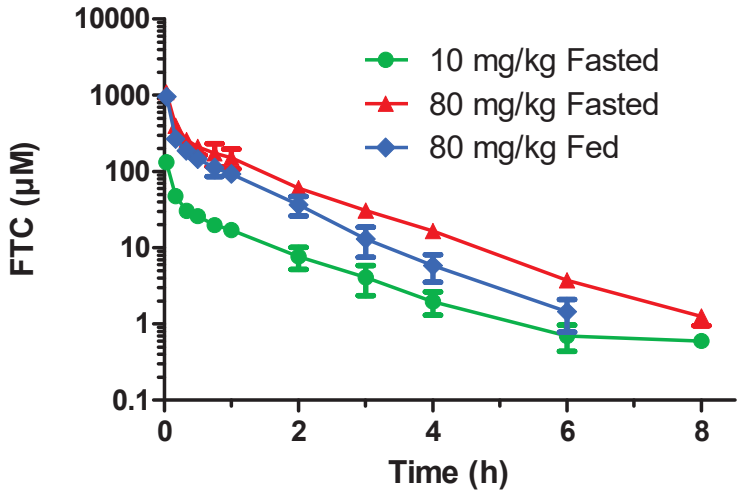
FTC = emtricitabine; SD = standard deviation
Source: Reports TEIN/93/0003 (10 mg/kg, n=5), TEIN/93/0004 (100 mg/kg, n=5) and [redacted]00101 (100 mg/kg, n=3)

3.2.1.2. FTC: Monkey, Oral/Intravenous

Two studies were performed with male cynomolgus monkeys dosed with a solution of FTC orally or by slow (2-minute) IV bolus administration. In the first study, 8 monkeys were administered single 10 mg/kg or 80 mg/kg doses of FTC (4 monkeys at each dose) in a crossover design study (m2.6.5, Section 3.1.4, TEZZ/93/0019). Animals were fasted overnight to 2 hours postdose. In a second study (m2.6.5, Section 3.1.5, [redacted]00301), 4 nonfasted monkeys were

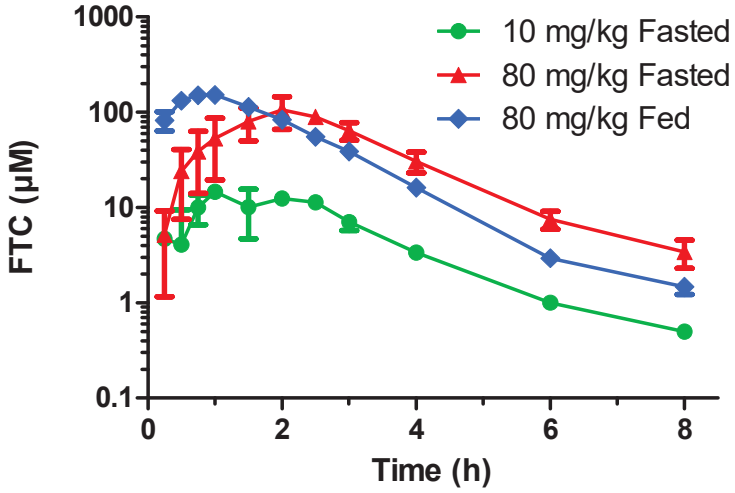
administered 80 mg/kg FTC intravenously or orally in a crossover design. Pharmacokinetic profiles are illustrated in Figure 3 and Figure 4. Pharmacokinetic parameters were similar for all 3 IV dose groups with V_{ss} values similar to those for total body water (0.77–0.80 L/kg) and moderate clearance values (0.70–0.97 L/kg). Half-lives were independent of dose and route of administration. In the fasted state, absorption was rapid and oral bioavailability values were 62.7% and 57.5% at 10 and 80 mg/kg, respectively. In the fed state, absorption was slower and the oral bioavailability was 97.4% at 80 mg/kg.

Figure 3. **Mean Plasma Concentration vs. Time Profile Following an Intravenous Bolus Dose of FTC in Solution to Male Cynomolgus Monkeys (mean ± SD, n = 4)**



FTC = emtricitabine; SD = standard deviation
Source: Reports TEZZ/93/0019 (fasted animals) and [redacted]00301 (fed animals)

Figure 4. **Mean Plasma Concentration vs. Time Profile Following an Oral Dose of FTC in Solution to Male Cynomolgus Monkeys (mean ± SD, n = 4)**



FTC = emtricitabine; SD = standard deviation
Source: Reports TEZZ/93/0019 (fasted animals) and [redacted]00301 (fed animals)

3.2.2. RPV: Single-Dose In Vivo Studies

3.2.2.1. RPV: Mouse, Oral

TMC278 base formulated in polyethylene glycol 400 (PEG400)/10% citric acid (CA) was administered once, by oral gavage, at 100, 400, and 1600 mg/kg to male and female CD-1 mice (m2.6.5, Section 3.2.2, TMC278-FK4259).

Due to the limited sampling points (1 and 6 hours), only an estimation of the AUC was possible. At 1600 mg/kg, estimated AUC_{0-6h} values were 307 and 287 µg·h/mL in males and females, respectively.

3.2.2.2. RPV: Rat, Oral/IV

TMC278 base was administered to male Sprague Dawley rats by oral gavage at 40 mg/kg formulated in PEG400 and at 40, 160, 400, and 800 mg/kg (males and females) formulated in PEG400/citric acid (CA) (10%) (m2.6.5, Sections 3.2.3 and 3.2.5, TMC278-FK4195 and TMC278-FK4278, respectively). TMC278 base formulated in PEG400/sterile water was administered intravenously to male Sprague Dawley rats at 4 mg/kg (m2.6.5, Sections 3.2.3, TMC278-FK4195). In addition, TMC278 was administered to male Sprague Dawley rats by oral gavage at 40 mg/kg as the base in PEG400 and as the HCl salt in Tween20/hydroxypropyl methylcellulose (HPMC)/water (m2.6.5, Sections 3.2.4, TMC278-NC106).

The absorption of TMC278 in PEG400 or PEG400/CA was rapid at the low dose (40 mg/kg) with C_{max} reached at 1 hour after dose administration. At higher doses, the plasma profiles showed a plateau and C_{max} values were only reached at 8 hours. $AUC_{0-\infty}$ values at 800 mg/kg were 86 and 233 µg·h/mL in males and females, respectively. AUC values increased dose-proportionally from 40 to 160 mg/kg and less than dose-proportionally from 160 to 800 mg/kg. At 800 mg/kg, exposure values (C_{max} and AUC) were up to 2.7-fold higher in females than in males.

The $t_{1/2}$ of TMC278 after IV administration was moderate (4.4 hours). The Vd_{ss} was 4.1 L/kg, which is approximately 6 times the total body water of rats (0.67 L/kg), indicating extensive distribution to the tissues. The total plasma clearance (Cl_p) was 1.3 L/h/kg, corresponding to a total blood clearance (Cl_b) of 1.9 L/h/kg (concentration blood/concentration plasma (C_b/C_{pl}) = 0.7 [m2.6.5, Sections 6.2.1, TMC278-NC112]), which is moderate compared to the hepatic blood flow of rats (3.3 L/h/kg). The absolute oral bioavailability (F_{abs}) was 32% and 39% after an oral dose of 40 mg/kg in the absence and presence of CA, respectively (compared to an IV dose of 4 mg/kg).

TMC278 in Tween20/HPMC/water gave a 26% lower exposure than the base in PEG400/CA, at an oral dose of 40 mg/kg.

3.2.2.3. RPV: Rabbit, IV

TMC278 base formulated in PEG400/water (25%) was administered intravenously to female New Zealand white rabbits at 1.25 (n=3), 2.9 (n=1), and 4 mg/kg (n=2) (m2.6.5, Sections 3.2.6, TMC278-FK4293).

After IV administration, AUC values increased dose-proportionally from 1.25 mg/kg to 4 mg/kg. Plasma levels declined slowly with a mean $t_{1/2}$ of 12 hours at 1.25 mg/kg and about 21 hours at the higher dose levels. The Vd_{ss} ranged between 0.32 and 0.56 L/kg, which is low compared to the total body water of rabbits (0.72 L/kg) indicating limited distribution. Total Cl_p was on average 0.03 L/h/kg, corresponding to a total Cl_b of 0.049 L/h/kg (C_b/C_{pl} = 0.61 [m2.6.5, Sections 6.2.1, TMC278-NC112]), which is very low compared to a hepatic blood flow of 4.3 L/h/kg.

3.2.2.4. RPV: Dog, Oral/IV

TMC278 base was administered to male beagle dogs by oral gavage at 5 mg/kg formulated in either PEG400 or PEG400/CA (10%) and intravenously at 1.25 mg/kg formulated in PEG400/water (25%) (m2.6.5, Sections 3.2.7, TMC278-FK4231). TMC278 base formulated in either PEG400 (40 and 80 mg/kg) or PEG400/CA (80 mg/kg) was also administered to male and female beagle dogs by oral gavage in a tolerance study (m2.6.5, Sections 4.2.15, TMC278-FK4102). In addition, TMC278 base formulated in PEG400/CA was administered by oral gavage to male beagle dogs at 5 mg/kg under fed and fasted conditions to investigate the food effect on the pharmacokinetics of TMC278 (TMC278-NC163 [FK5141]).

The plasma profiles of TMC278 in PEG400 or PEG400/CA showed a plateau after an initial rapid absorption until at least 7 to 8 hours postdose. At 40 mg/kg in PEG400, $AUC_{0-\infty}$ values were 41 and 52 µg.h/mL in the male and female dog, respectively. Between 40 and 80 mg/kg in PEG400, no further increase in exposure was seen. Mean C_{max} and AUC values increased substantially at 5 mg/kg with the addition of CA to the formulation (~ 3-fold). At 80 mg/kg, only C_{max} increased with addition of CA while AUC values were comparable.

TMC278 was slowly eliminated following IV administration with a $t_{1/2}$ of 31 hours. The Vd_{ss} was 5.2 L/kg, which is about 9 times the total body water of dogs (0.604 L/kg), indicating extensive distribution to tissues. Total Cl_p was on average 0.14 L/h/kg, corresponding to a total Cl_b of 0.20 L/h/kg (C_b/C_{pl} = 0.69 [m2.6.5, Sections 6.2.1, TMC278-NC112]), which is very low compared to a hepatic blood flow of 1.9 L/h/kg.

The F_{abs} was 31% and 80% after an oral dose of 5 mg/kg in the absence and presence of CA, respectively (as compared to an IV dose of 1.25 mg/kg).

The effect of food was tested in dogs at 5 mg/kg of TMC278 formulated in PEG400/CA. Under fed conditions, the absorption rate of TMC278 was slower but the extent of absorption was similar, leading to a lower C_{max} without any change in AUC.

3.2.2.5. RPV: Monkey, Oral

TMC278 base was administered to male cynomolgus monkeys by oral gavage at 5 mg/kg formulated in PEG400/CA (10%) and intravenously at 1.25 mg/kg formulated in PEG400/water (25%) (m2.6.5, Sections 3.2.9, TMC278-DM02403). TMC278 formulated in aqueous HPMC (1%)/Tween 20 (0.5%) was administered to female cynomolgus monkeys at 250 mg/kg twice daily (BID; dosing interval of 8 hours) and at 500 mg/kg once daily (m2.6.5, Sections 4.2.22, TMC278-NC326).

After once-daily administration, the inter-individual variability was high particularly for C_{max} (CV = 71%). C_{max} occurred between 2 and 24 hours after oral administration. The $t_{1/2}$ after IV administration was 7.1 hours. The Vd_{ss} was 4.2 L/kg, which is about 6 times the total body water of monkeys (0.692 L/kg), indicating that TMC278 is widely distributed to tissues. Total Cl_p was on average 0.93 L/h/kg, corresponding to a total Cl_b of 0.98 L/h/kg (C_b/C_{pl} = 0.95 [m2.6.5, Sections 6.2.2, TMC278-NC332]), which is low compared to a hepatic blood flow of 2.62 L/h/kg. At 5 mg/kg, $AUC_{0-\infty}$ was 1.3 µg.h/mL.

The F_{abs} was 24% after an oral dose of 5 mg/kg (compared to an IV dose of 1.25 mg/kg).

A higher exposure (AUC) to TMC278 was observed after a BID dose of 250 mg/kg of TMC278 compared to a single dose of 500 mg/kg. However, this was not confirmed in the subsequent repeat-dose study. At 250 mg/kg BID, the $AUC_{0-\infty}$ value was 10.1 µg·h/mL.

3.2.3. TAF and TFV: Single-Dose In Vivo Studies

3.2.3.1. TAF: Mouse, Oral

The mouse plasma pharmacokinetic studies were conducted by dosing either GS-7340-02 or GS-7340-03 to male CD-1 mice or GS-7340-03 to both male and female 001178-W mice (m2.6.5, Section 3.3.2, AD-120-2014 and m2.6.5, Section 3.3.3, AD-120-2016). Although TAF was observed in mouse plasma in a dose dependent manner, the concentrations were limited and $t_{½}$ could not be determined. Tenofovir exposure increased with the increase in dose and was greater than dose proportional between 10 to 100 mg/kg. Gender differences in plasma TFV levels were less than 2-fold in C_{max} and AUC_{0-t} values. The pharmacokinetic profiles for the 2 different fumarate forms were generally similar. Pharmacokinetic parameters for GS-7340-02 and GS-7340-03 are summarized in Table 5. Values for C_{max} and AUC_{0-t} for TFV were generally similar between GS-7340-02 and GS-7340-03. Together with the rat pharmacokinetics results, the 2 different forms of TAF do not affect the pharmacokinetic properties. The pharmacokinetic parameters following a single dose of GS-7340-03 to 001178-W wild type mice are summarized in Table 6. Measurable concentrations were limited for TAF. No consistent gender-based differences were observed in TAF C_{max} and AUC_{0-t} values. Tenofovir alafenamide was extensively converted to TFV in 001178-W mice following oral gavage administration of GS-7340-03. Exposure to TFV increased with the increase in dose level from 10 to100 mg/kg. The increases in C_{max} and AUC_{0-t} were greater than dose proportional between the 10 to 100 mg/kg. Gender differences in plasma concentration of TFV were less than 2-fold in C_{max} and AUC_{0-t} values.

Table 5. Dose Dependent Plasma Pharmacokinetic Parameters Following a Single Oral Administration of GS-7340-02 and GS-7340-03 to Male CD-1 Mice

Test Article	GS-7340-02						GS-7340-03					
Dose (mg/kg)	10		30		100		10		30		100	
Analyte	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV
C_{max} (µg/mL)	5.53	106	NA	440	37.1	1827	NA	85.4	10.3	383	34.7	2152
t_{max} (h)	0.083	0.50	NA	0.25	0.083	0.75	NA	0.50	4.00	0.50	0.25	1.50
$t_{½}$ (h)	NA	NA	NA	NA	NA	NA	NA	5.16	NA	10.1	NA	NA
AUC_{0-t} (ng·h/mL)	NA	455	NA	2005	26.0	10643	NA	493	NA	2477	11.3	10866

NA = not applicable
Source: AD-120-2014

Table 6. Dose Dependent Plasma Pharmacokinetic Parameters Following a Single Oral Administration of GS-7340-03 to 001178-W Wild Type Mice

Dose (mg/kg)	10				30				100			
Analyte	TAF		TFV		TAF		TFV		TAF		TFV	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
C_{max} (ng/mL)	NA	NA	175	100	8.80	117	615	421	648	280	1988	1733
t_{max} (h)	NA	NA	0.25	0.50	0.083	0.5	0.25	0.25	0.25	0.50	0.50	0.50
$t_{½}$ (h)	NA	NA	9.78	8.20	NA	NA	9.51	10.9	NA	NA	8.04	11.0
AUC_{0-t} (ng·h/mL)	NA	NA	735	354	NA	NA	2639	2053	194	104	10026	7131

NA = not applicable
Source: AD-120-2016

3.2.3.2. TAF: Rat, Oral

Plasma pharmacokinetic studies following a single oral administration of TAF were performed to determine pharmacokinetic parameters for TFV, to assess potential differences between GS-7340-02 and GS-7340-03, and to compare exposure to TFV between TAF and TDF (m2.6.5, Section 3.3, R990130, AD-120-2015, and R2000065). In all cases TAF was rapidly absorbed and converted to TFV. The plasma TFV t_{max} was less than 1 hour. Tenofovir exposure increased with the increase in dose and was greater than dose proportional between 5 to 100 mg/kg. No significant differences in pharmacokinetic profiles were observed between GS-7340-02 and GS-7340-03 (Table 7). The plasma C_{max} and AUC for TFV were 2- to 3-fold higher when Sprague-Dawley rats were orally dosed with 400 mg/kg TAF compared to 400 mg/kg TDF.

Table 7. Dose Dependent Plasma Pharmacokinetic Parameters following a Single Oral Administration of GS-7340-02 and GS-7340-03 to Male Sprague-Dawley Rats

Test Article	GS-7340-02			GS-7340-03		
Dose (mg/kg)	**5**	**25**	**100**	**5**	**25**	**100**
Analyte	**TFV**	**TFV**	**TFV**	**TFV**	**TFV**	**TFV**
C_{max} (µg/mL)	32.5	199	1240	39.3	364	1670
t_{max} (h)	0.667	0.583	0.833	0.583	0.833	0.667
$t_{½}$ (h)	NA	11.2	10.3	NA	7.89	7.85
AUC_{0-t} (ng·h/mL)	122	1395	7771	88.5	1810	9759

3.2.3.3. TAF: Dog, Oral and IV

In order to assess the effects of the stereo configuration, fumarate form, food, and the route of administration, the plasma and PBMC pharmacokinetic profiles were determined in Beagle dogs following IV bolus (GS-7340-02 [6.3 mg/kg]), or oral administration (TAF as free base [18.0 mg/kg], its diastereomer GS-7339 [18.0 mg/kg], the mixture GS-7171 [16.0 mg/kg], or GS-7340-02 [4,8, 5.0, and 20 mg/kg under fasted and 5.0 mg/kg under fed conditions]) (m2.6.5, Section 3.3.7, 99-DDM-1278-001-PK). Following oral administration, TAF and its diasteroisomer were rapidly absorbed and eliminated with a t_{max} of less than 0.5 h and $t_{½}$ ranging from 0.2-0.9 h. The plasma exposures to the intact prodrugs were similar when TAF or GS-7339 were dosed separately, however, when the isomeric mixture, GS-7171, was dosed, the exposure to GS-7339 was approximately 3-fold higher than TAF. The TFV exposure in plasma was similar for both diastereomers. The TFV exposure in PBMCs was approximately 4-fold higher when animals were dosed with TAF than GS-7339. All the pharmacokinetic parameters were similar for the free base and fumarate form. Plasma exposures to TAF and TFV and PBMC exposure to TFV were approximately 2.5-fold higher in fasted dogs than in fed dogs.

The plasma and liver pharmacokinetic profiles were determined following a single oral dose of 10 mg/kg TAF to male Beagle dogs (m2.6.5, Section 3.3.8, AD-120-2034). Tenofovir alafenamide was rapidly absorbed and eliminated with observed plasma t_{max} of 0.08 hours and $t_{½}$ of 0.24 hours. Tenofovir was the major metabolite found in plasma with a C_{max} value of 2.23 µM. The pharmacologically active metabolite, TFV-DP was the major metabolite in liver achieving a C_{max} of 126 µM at 4.0 hours postdose.

3.2.3.4. TAF: Monkey, Oral

The plasma pharmacokinetic profiles for TAF and TFV and TFV concentrations in PBMCs were determined in rhesus monkeys following a single oral dose of GS-7340-02 at 0.5, 5.0, and 50 mg/kg (m2.6.5, Section 3.3.9, P2000087). The plasma pharmacokinetic parameters are summarized in Table 8. Tenofovir alafenamide and TFV levels increased rapidly with t_{max} vales of approximately 0.5 and 1 hour, respectively. The TFV levels in PBMCs were determined

before and after treatment with acid phosphatase, which was used to convert the phosphorylated metabolites to TFV (Table 9). Tenofovir persisted in PBMCs up to 96 h with an apparently slower decline in PBMCs than in plasma. The TFV levels were significantly higher in the samples treated with acid phosphatase suggesting that a significant proportion of TFV-related material in PBMCs was in phosphorylated forms.

Table 8. Dose Dependent Plasma Pharmacokinetic Parameters for TAF And TFV Following a Single Oral Administration of GS-7340-02 to Rhesus Monkeys

GS-7340-02 Dose (mg/kg)	0.5	5	50	0.5	5	50
Analyte	**TAF**			**TFV**		
C_{max} (ng/mL)	2.79	125	4143	7.72	161	1326
t_{max} (h)	0.38	0.8	0.5	1	1.33	1.0
$t_{½}$ (h)	0.61	0.23	0.40	4.62	9.92	17.33
$AUC_{0\text{-last}}$ (ng·h/mL)	1.22	95.1	3811	39.9	1037	9934
$AUC_{0\text{-}\infty}$ (ng·h/mL)	2.47	80.0	3846	52.7	1069	10250

Table 9. Concentrations of TFV in PBMCs from Monkeys Following a Single Oral Administration of GS-7340-02 at 5 and 50 mg/kg

	TFV PBMC Levels (ng/10^6 Cells)			
	Without Phosphatase Treatment		With Phosphatase Treatment	
GS-7340-02 Dose (mg/kg)	5	50	5	50
2 h	0.47	17.0	0.73	34.2
24 h	0.06	6.82	0.62	20.1
96 h	BLQ	3.03	0.18	8.68

3.3. Repeat-Dose In Vivo Studies

3.3.1. FTC: Repeat-Dose In Vivo Studies

Multiple dose in vivo toxicokinetic studies were performed in mouse, rat, and monkey in support of safety evaluation. The results are presented in detail in m2.6.6, Section 3.1 and general conclusions from representative studies are noted below.

3.3.1.1. FTC: Mouse, Oral

Toxicokinetic data have been generated from a number of short- to long-term studies in mice, following 14-day to 6-month dosing of FTC with doses ranging from 0 to 3000 mg/kg/day. The systemic exposure of FTC at steady state increased proportionally with dose administered (m2.6.7, Section 7.1, [redacted]00701, TOX599, TOX022 [and TOX022 pharmacokinetic report], TOX628).

In a 2-year oral oncogenicity study, FTC was administered once daily to CD-1 mice by oral gavage at doses of 0, 80, 250, and 750 mg/kg/day (m2.6.5, Section 4.1.1, TOX-109). Emtricitabine was rapidly absorbed following all doses with peak plasma concentrations occurring 0.5 to 1.0 hour postdose. C_{max} and $AUC_{0–24}$ results are summarized in (TOX-109). $AUC_{0–24}$ and C_{max} increased proportionally with dose over the range of 80 to 750 mg/kg/day. In general, the exposures ($AUC_{0–24}$) in male mice were similar to those in female mice at all doses. $AUC_{0–24}$ and C_{max} values were higher on Week 26 compared to Week 2.

3.3.1.2. FTC: Rat, Oral

Toxicokinetic data from a subchronic study in rats with FTC doses showed linear relationship between systemic exposure and daily dose of FTC from 120 to 3000 mg/kg (m2.6.7, Section 7.1.5, TOX097).

In a 2-year oral oncogenicity study, FTC was administered once daily to Sprague-Dawley rats by oral gavage at doses of 0, 60, 200, and 600 mg/kg/day. Emtricitabine was rapidly absorbed following all doses with peak plasma concentrations occurring at 0.5 hours postdose. C_{max} and $AUC_{0–24}$ results are summarized in m2.6.5, Section 4.1.2 (TOX-108). AUC_{0-24} and C_{max} increased with dose over the range of 60 to 600 mg/kg/day. In general, exposure ($AUC_{0–24}$) in male rats was similar to those in female rats. $AUC_{0–24}$ and C_{max} values were higher on Week 26 compared to Week 2.

3.3.1.3. FTC: Monkey, Oral

A 1-month toxicology study of FTC was conducted in cynomolgus monkeys at oral doses of 0, 80, 400, and 2000 mg/kg/day, given in 2 divided doses, 6 hours apart (m2.6.7, Section 7.1.6, TOX600 and Addendum report). Plasma concentrations of FTC were measured in samples drawn predose and over the first 6 hours after the first dose on Days 3 and 27. Cerebrospinal fluid (CSF) and corresponding plasma samples were obtained for analysis at 1 hour postdose on Day 28. There were no significant differences in drug levels in plasma and CSF between males and females. No significant differences in pharmacokinetic parameters were determined between Day 3 and Day 28. Mean C_{max} values increased with dose and AUC_{0-6} were proportional to the dose. The overall mean (combined male, female and dose day) C_{max} values were 13.9, 62.8, and 198 μg·h/mL for monkeys given 40, 200, and 1000 mg/kg/dose, respectively. The overall AUC_{0-6} were 13.9, 62.8, and 198 μg·h/mL for monkeys given 40, 200, and 1000 mg/kg/dose, respectively. The concentration of FTC in CSF 1 hour after dosing on Day 28 averaged 3.9 ± 0.7 percent of the corresponding plasma levels.

A 3-month oral toxicity study was performed with FTC in cynomolgus monkeys (m2.6.7, Section 7.1.7, TOX627). The doses tested were 0, 40, 200, and 1000 mg/kg/day (n = 5/sex/group), given as 2 divided doses by nasogastric intubation, with approximately 6 hours between doses. Emtricitabine was rapidly and well absorbed with peak plasma concentrations occurring between 0 and 2 hours. No significant differences were seen between results from males and females at any dose level and there were no significant changes in pharmacokinetic parameters between dose Days 3 and 87. Maximum plasma levels of FTC increased with dose, but increased linearly only between the 40 and 200 mg/kg/day doses.

The overall mean (combined male, female and dose day) C_{max} values were 5.63, 25.2, and 101 µg·h/mL for monkeys given 40, 200, and 100 mg/kg/day, respectively. The AUC_{0-6} values were proportional to the dose. The overall AUCs were 13.3, 60.8, and 310 µg·h/mL for monkeys given 40, 200, and 1000 mg/kg/day, respectively. Similar AUCs across multiple days suggest that there was no significant accumulation of FTC over the dosing period.

A 1-year oral toxicity study was performed with FTC in cynomolgus monkeys (m2.6.7, Section 7.1.8, TOX032). The doses tested were 0, 50, 200, and 500 mg/kg/day, each given as 2 divided doses by nasogastric intubation, with approximately 5 hours between doses. Emtricitabine was rapidly and well absorbed with peak plasma concentrations occurring between 0.5 to 2 hours after dosing. Plasma FTC was eliminated with a terminal $t_{½}$ of 2 to 4 hours at all dose levels. The $t_{½}$ estimates did not change after multiple-dose administration. There were no major differences in plasma FTC exposure between male and female monkeys. Slightly higher plasma exposures to FTC were achieved at Weeks 13, 26, and 52 as compared to Day 0 for each dose level. Plasma C_{max}, $AUC_{0–6}$, and estimated steady-state $AUC_{0–24}$ (Weeks 13, 26, and 52) increased linearly with the dose administered over the range of 50 to 500 mg/kg/day in both male and female monkeys.

3.3.2. RPV: Repeat-Dose In Vivo Studies

3.3.2.1. RPV: Mouse, Oral

TMC278 formulated in aqueous HPMC (0.5%) was administered to male and female CD-1 mice by oral gavage in a 3-month study (20, 80, and 320 mg/kg/day) and a carcinogenicity study (20, 60, and 160 mg/kg/day) (m2.6.5, Sections 4.2.3 and 4.2.4, TMC278-NC119 and TMC278-NC120, respectively). TMC278 was administered to male and female CD-1 mice by dietary admixture at 40 and 400 mg/kg/day and by oral gavage at 400 mg/kg/day formulated in aqueous HPMC (0.5%) in a 14-day study (m2.6.5, Section 4.2.1, TMC278-NC118). In addition, TMC278 formulated in aqueous HPMC (0.5%) was administered to male and female CB6F1-nonTgrasH2 transgenic mice in a 1-month study (20, 80, and 320 mg/kg/day) (m2.6.5, Section 4.2.2, TMC278-NC121).

In all these studies, after oral gavage administration of TMC278, peak plasma levels of TMC278 were generally reached between 0.5 and 2 hours after dosing, indicating rapid absorption. At 20 mg/kg/day TMC278, plasma concentrations declined rapidly after peak time. At higher dose levels (up to 320 mg/kg/day), the plasma profiles showed a plateau after an initial rapid absorption resulting in constant or even increasing plasma levels until at least 8 hours postdose (Figure 5). Plasma peak concentrations increased less than dose-proportionally. $AUC_{0–\infty}$ values increased dose-proportionally or slightly less than dose-proportionally in males and females at Day 1. After repeat-dose administration, $AUC_{0–24h}$ values increased less than dose-proportionally in males and dose-proportionally to more than dose-proportionally in females.

At low dose levels (20 mg/kg/day in females and up to 160 mg/kg/day in males), exposure after repeated administration was comparable to that at Day 1. In females, at higher dose levels (60 to 320 mg/kg/day) exposure after repeated administration was higher (up to 2.2-fold) than at Day 1. In males, at 320 mg/kg/day, exposure after repeated administration was lower (up to 34% at Day 87) compared to Day 1 (Table 10).

Overall, exposure in females was similar to the exposure in males, except at 160 and 320 mg/kg/day after repeated administration, where exposure in females was higher than in males (up to 2-fold).

Exposure ($AUC_{0\text{-}24h}$) to TMC278 after dietary administration at 400 mg/kg/day was similar in females and somewhat higher (1.6-fold) in males when compared to oral gavage treatment.

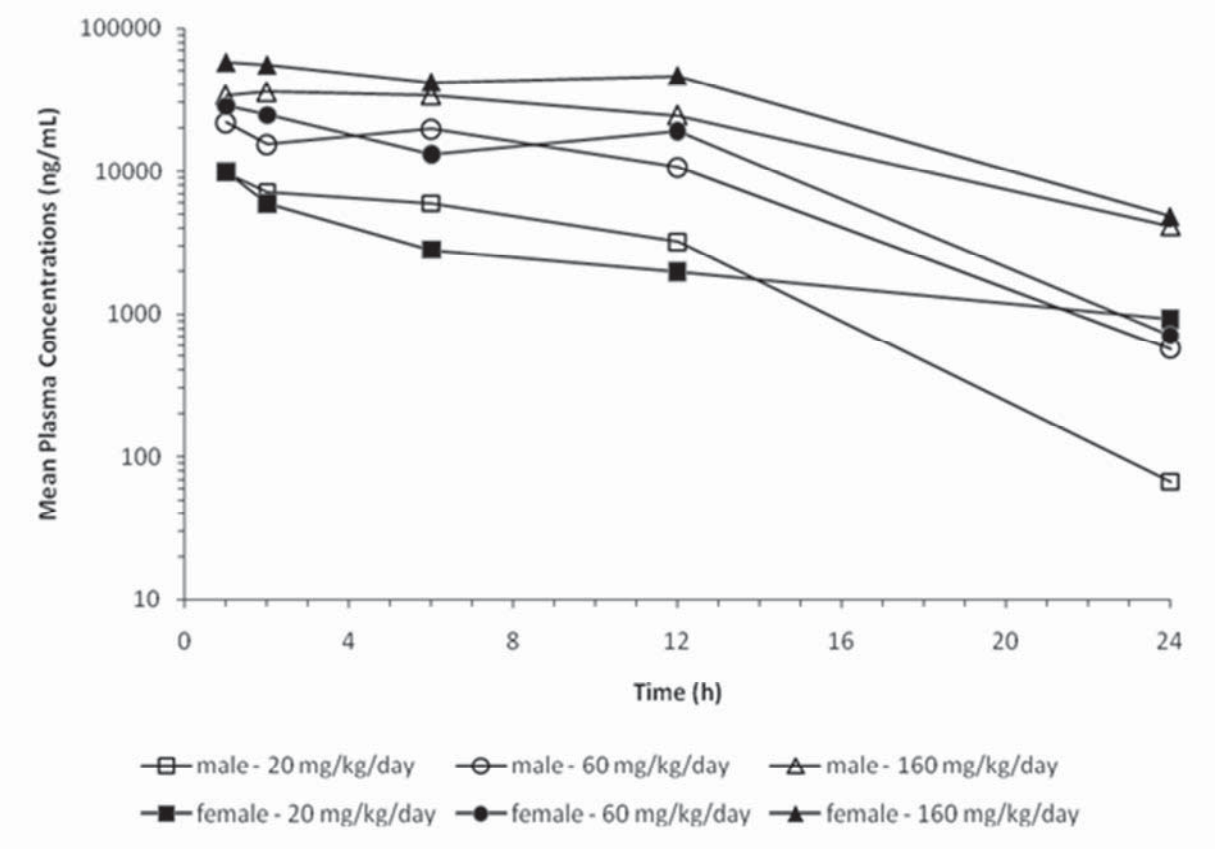
After repeated administration of TMC278, exposure to TMC278 in CB6F1-nonTgrasH2 transgenic mice was similar to that observed in CD-1 mice for both C_{max} and AUC.

Table 10. Pharmacokinetic Parameters of TMC278 After Single or Repeated Administration by Oral Gavage of TMC278 in Mice

Study	Dose Level (mg/kg/day)	Sampling Time (Day)	C_{max} (µg/mL)		AUC[a] (µg·h/mL)	
			Male	Female	Male	Female
3-month study in CD-1 mice (TMC278-NC119)	20	1	14	13	71	59
		31	14	18	61[b]	74
		87	18	19	80	61
	80	1	28	32	236	250
		31	38	37	263	313
		87	34	42	210	313
	320	1	63	55	1010	707
		31	63	84	860	1170
		87	61	90	665	1360
24-month study in CD-1 mice (TMC278-NC120)	20	1	9.9	13	60	61
		Week 28	9.8	9.9	76	51
	60	1	23	24	239	182
		Week 28	22	29	230	278
	160	1	41	38	440	345
		Week 28	36	58	505	766

a $AUC_{0-\infty}$ after single dose and AUC_{0-24h} after repeated dose
b AUC_{0-8h}

Figure 5. Plasma Concentrations of TMC278 in CD-1 Mice After Repeated Administration of TMC278 by Oral Gavage Once Daily for 28 Weeks in the Carcinogenicity Study



3.3.2.2. RPV: Rat, Oral

In a pilot study, TMC278 base formulated in PEG400 was administered by oral gavage to male rats in a 5-day toxicity study (40 and 400 mg/kg/day) (m2.6.5, Section 4.2.5, TMC278-FK4103). TMC278 base formulated in PEG400/CA (10%) was administered by oral gavage to male and female rats in a 2-week toxicity study (40, 120, and 400 mg/kg/day), a 1-month toxicity study (10, 40, and 160 mg/kg/day), and a 6-month toxicity study (40, 120, and 400 mg/kg/day) (m2.6.5, Section 4.2, TMC278-FK4243, TMC278-TOX5692, and TMC278-NC101). TMC278 formulated in aqueous HPMC (0.5%) was administered by oral gavage to male and female rats in a 2-week toxicity study (400, 1500, and 2000 mg/kg/day) (m2.6.5, Section 4.2.8, TMC278-NC177) and a carcinogenicity study (40, 200, 500, and 1500 mg/kg/day) (m2.6.5, Section 4.2.12, TMC278-NC123). In a 1-month bridging study, TMC278 base formulated in PEG400/CA (10%) and TMC278 formulated in aqueous HPMC (0.5%) were administered by oral gavage to male and female rats (10 and 400 mg/kg/day) (m2.6.5, Section 4.2.10, TMC278-NC117). TMC278 (400 and 1200 mg/kg/day) and TMC278 base (400 mg/kg/day) were also administered by dietary admixture to male and female rats in a 2-week toxicity study. In the same study, TMC278 formulated in aqueous HPMC (0.5%) was administered by oral gavage at 400 mg/kg/day (m2.6.5, Section 4.2.7, TMC278-NC136).

In addition, TMC278 base formulated in PEG400/CA (10%) was administered by oral gavage to pregnant rats in an embryo-fetal toxicity study from gestation day (GD) 6 to GD 16 (40, 120, and 400 mg/kg/day) (m2.6.5, Section 7.2.1, TMC278-NC105). TMC278 formulated in aqueous HPMC (0.5%) was also administered to juvenile rats of 12-days-old by oral gavage for 14 days (m2.6.5, Section 4.2.13, TMC278-NC168). Sprague Dawley rats were used in all the above-mentioned toxicity studies.

After oral administration of TMC278 base for 5 days using PEG400 (40 and 400 mg/kg/day), $AUC_{0-\infty}$ values at Day 1 were similar at 40 mg/kg and lower at 400 mg/kg (up to 70%) compared to PEG400/CA.

After oral gavage administration of TMC278 base formulated in PEG400/CA, C_{max} was reached within 4 hours after administration at low doses (up to 40 mg/kg/day), whereas at higher dose levels (up to 400 mg/kg/day) plasma profiles showed a plateau between 2 and 10 hours. Exposure, expressed as C_{max} and AUC, generally increased less than dose-proportionally at Day 1 and after repeated administration in both males and females (Figure 6). In females, C_{max} and AUC_{0-24h} values increased slightly (up to 1.6-fold) with repeated administration, while in males, particularly at high doses, exposure decreased slightly (up to 40%) (Table 11). In all studies, exposure in females appeared to be higher (2- to 4-fold) than in males at Day 1 and after repeated administration.

After oral gavage administration of TMC278 formulated in aqueous HPMC, C_{max} was reached between 2 and 8 hours. Exposure to TMC278, expressed as C_{max} and AUC, increased less than dose-proportionally at Day 1 and after repeated administration in both males and females up to 1500 mg/kg/day. Between 1500 and 2000 mg/kg/day no further increase in exposure was seen in males, whereas an increase (1.3-fold) was seen in females. In males, C_{max} and AUC_{0-24h} values decreased after repeated administration particularly in comparison to Day 1 in the carcinogenicity study where the decrease was between 67% and 76% at all doses. However, no further decrease in exposure (C_{max} and AUC) was seen between Week 27 and Week 39. In females, no clear tendency was observed across studies. For example, in the carcinogenicity study, exposure at Week 39 was similar to Day 1 at 200 and 1500 mg/kg/day and was slightly lower (up to 27%) at 40 and 500 mg/kg/day. C_{max} and AUC tended to be similar or higher (2-fold) in females than in males at Day 1, whereas after repeated dose, values in females were 1.3- to 5-fold higher.

In a 1-month bridging study comparing oral administration of TMC278 in aqueous HPMC with TMC278 base in PEG400/CA, AUC values after the base were comparable to those after the salt at the low dose (10 mg/kg/day). At 400 mg/kg/day, AUC_{0-24h} values at Day 24 after the base were 1.7- to 2.7-fold higher than those after the salt.

After repeated dietary administration, exposure to TMC278 at 400 mg/kg/day was slightly (1.3- to 1.6-fold) higher for TMC278 and similar for TMC278 base in both males and females compared to oral gavage treatment.

In pregnant rats, following daily oral administration of TMC278 base (PEG400/CA) at 40, 120, and 400 mg/kg/day from GD 6 to GD 16, exposures were similar to the exposures in nonpregnant females (see Table 11). Exposure, expressed as C_{max} and AUC, increased less than dose-proportionally and was approximately the same from GD 6 to GD 16.

In a dose range-finding pre- and postnatal development study in rats, TMC278 in aqueous HPMC was administered at 40, 120, and 400 mg/kg/day to dams from Day 6 of gestation to Day 7 of lactation. The same dose levels were also administered directly to the pups from Day 12 until Day 25 of age. On Day 7 of lactation, exposure ($AUC_{0–24h}$) in pups via milk was 0.62 and 0.74 µg·h/mL at 40 mg/kg, 0.94 and 0.91 µg·h/mL at 120 mg/kg, and 1.9 and 1.8 µg h/mL at 400 mg/kg/day in males and females, respectively. In pups, exposure to TMC278 via milk increased with the dose, but not dose-proportionally.

After repeated direct administration to pups for 14 days, exposure increased less than dose-proportionally. There was no difference in exposure between male and female pups in contrast to the adult rats (see Table 11).

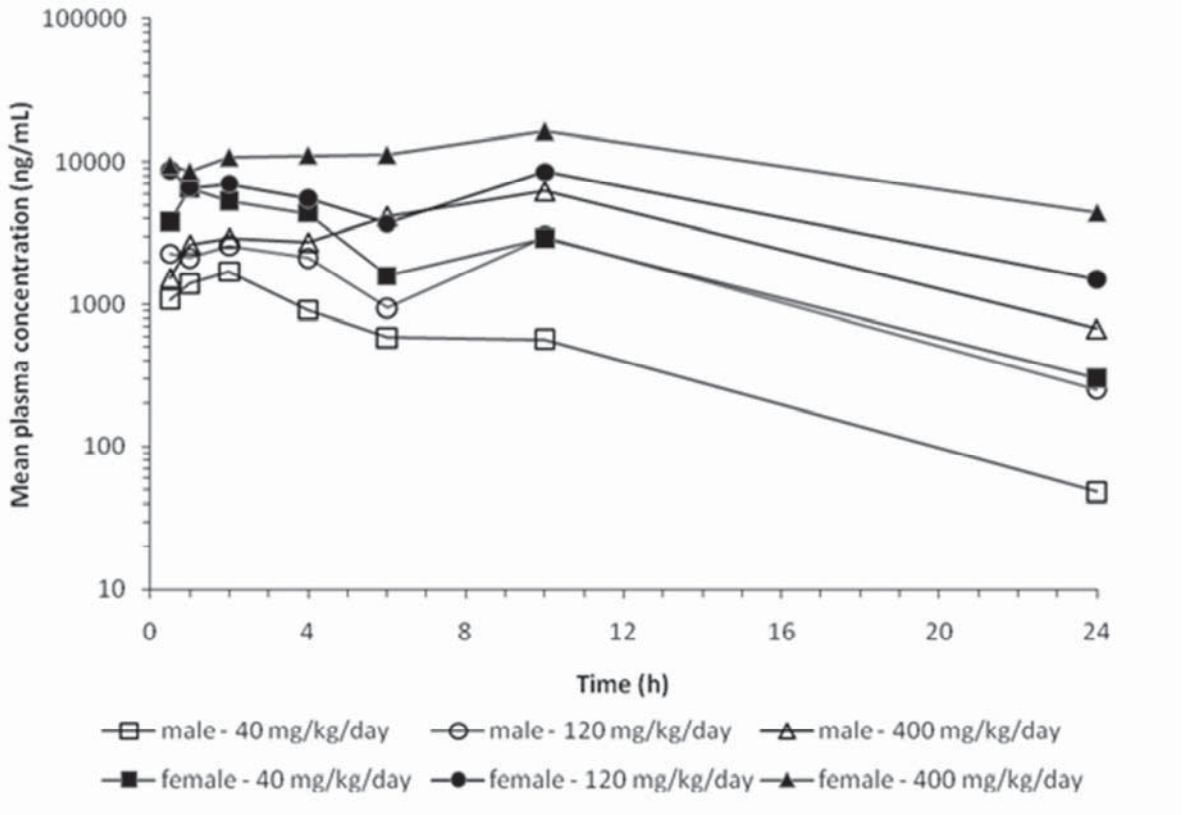
Table 11. Pharmacokinetic Parameters of TMC278 After Single or Repeated Administration by Oral Gavage of TMC278 Base or TMC278 in Sprague Dawley Rats

Study	Formulation	Dose Level (mg/kg/day)	Sampling Time (Day)	C_{max} (µg/mL)		AUC[a] (µg·h/mL)	
				Male	Female	Male	Female
6-month study in rats (TMC278-NC101)	TMC278 base in PEG400/CA (10%)	40[b]	1	2.9	6.5	19	32
			175	1.7	6.6	12	50
		120[b]	1	6.4	8.5	53	83
			175	3.0	8.8	35	116
		400[b]	1	9.1	17	92	160
			175	6.2	16	73	244
Carcinogenicity study in rats (TMC278-NC123)	TMC278 in HPMC (0.5%)	40	1	1.6	2.2	19	17
			Week 39	0.82	2.1	6.3	14
		200	1	2.6	4.2	34	40[c]
			Week 39	1.3	4.7	8.2	41
		500	1	4.5	6.5	45	63
			Week 39	1.8	8.5	14	46
		1500	1	6.1	7.0	58	81
			Week 39	2.2	9.4	18	84
Embryo-fetal study in rats (TMC278-NC105)	TMC278 base in PEG400/CA (10%)	40	1 (GD6)	—	4.9	—	33
			11 (GD16)	—	5.6	—	37
		120	1 (GD6)	—	6.0	—	65
			11 (GD16)	—	7.2	—	63
		400	1 (GD6)	—	14	—	182
			11 (GD16)	—	13	—	152

Study	Formulation	Dose Level (mg/kg/day)	Sampling Time (Day)	C_{max} (µg/mL)		AUC[a] (µg·h/mL)	
				Male	Female	Male	Female
Pre- and postnatal study in rats: administration to pups (TMC278-NC168)	TMC278 in HPMC (0.5%)	40[d]	Day 25 of age	2.6	5.8	12	18
		120[d]	Day 25 of age	3.7	3.6	34	28
		400[d]	Day 25 of age	9.1	7.3	50	53

a $AUC_{0-\infty}$ after single dose and AUC_{0-24h} after repeated dose
b total dosing volume of 10 mL/kg was changed after Day 83 and split in 2 administrations of 5 mL/kg each, with 1.5 hours between the 2 administrations
c AUC_{0-24h}
d Dose administered to pups from Day 12 to Day 25 of age.
CA: citric acid; GD: gestation day; PEG400: polyethylene glycol 400; HPMC: hydroxypropyl methyl cellulose.

Figure 6. Plasma Concentrations of TMC278 in Sprague Dawley Rats After Repeated Administration of TMC278 Base by Gavage Once Daily for 6 Months (Day 175)



3.3.2.3. RPV: Rabbit, Oral

TMC278 base formulated in aqueous HPMC (0.5%) was administered by oral gavage to nonpregnant New Zealand white rabbits in a 5-day toxicity study (100, 300, and 1000 mg/kg/day) and to pregnant rabbits in a pilot (25, 75, and 150 mg/kg/day) and a main (5, 10, and 20 mg/kg/day) embryo-fetal developmental toxicity study from GD 6 to GD 19 (m2.6.5, Sections 4.2.14, 7.2.2, and 7.2.3, TMC278-NC126, TMC278-NC128, and TMC278-NC130, respectively).

After oral gavage administration for 5 days of TMC278 base in nonpregnant rabbits and for 2 weeks in pregnant rabbits, C_{max} were reached between 7 and 19 hours after dosing. In the main embryo-fetal developmental study, mean C_{max} and $AUC_{0–24h}$ values at GD 19 were 15 µg/mL and 232 µg·h/mL at 20 mg/kg/day, respectively. C_{max} and AUC_{0-24h} values increased less than dose-proportionally in pregnant and nonpregnant rabbits from 5 to 1000 mg/kg/day. The exposure values observed following 2 weeks of repeated dosing were similar to those observed at Day 1.

3.3.2.4. RPV: Dog, Oral

In a pilot study, TMC278 base formulated in PEG400/CA (10%) was administered by oral gavage to a male and a female beagle dog in a 5-day toxicity study (80 mg/kg/day) (m2.6.5, Section 4.2.15, TMC278-FK4102). TMC278 base formulated in PEG400/CA (10%) was administered by oral gavage to male beagle dogs in a 7-day toxicity study (20, 40, and 80 mg/kg/day) and at 5, 10, and 40 mg/kg/day to male and female beagle dogs in a 1-month toxicity study, a 6-month toxicity study, and a 12-month toxicity study (m2.6.5, Sections 4.2.16, 4.2.17, 4.2.19, and 4.2.20, TMC278-FK4244, TMC278-TOX5650, TMC278-NC115, and TMC278-NC107, respectively). In addition, TMC278 base formulated in PEG400/CA (10%) and TMC278 formulated in aqueous HPMC (0.5%) were administered by oral gavage to male and female beagle dogs (5 and 40 mg/kg/day) in a 1-month bridging study (m2.6.5, Section 4.2.18, TMC278-NC116).

In all studies with TMC278 base formulated in PEG400/CA, the rate of absorption was highly variable based on mean time to reach C_{max} (t_{max}) values ranging between 1.3 and 24 hours. The plasma concentration-time profile showed a plateau (see Figure 7). In general, exposure expressed as mean C_{max} and AUC_{0-24h} increased less than dose-proportionally in both males and females (Table 12). No further increase in exposure was seen between 40 and 80 mg/kg/day. Exposure (AUC) increased after repeated administration as compared to Day 1 mainly due to the long terminal $t_{1/2}$ of TMC278. No clear difference in pharmacokinetics was seen between males and females.

In general, a high inter-study variability was seen particularly at 40 mg/kg/day after repeated administration.

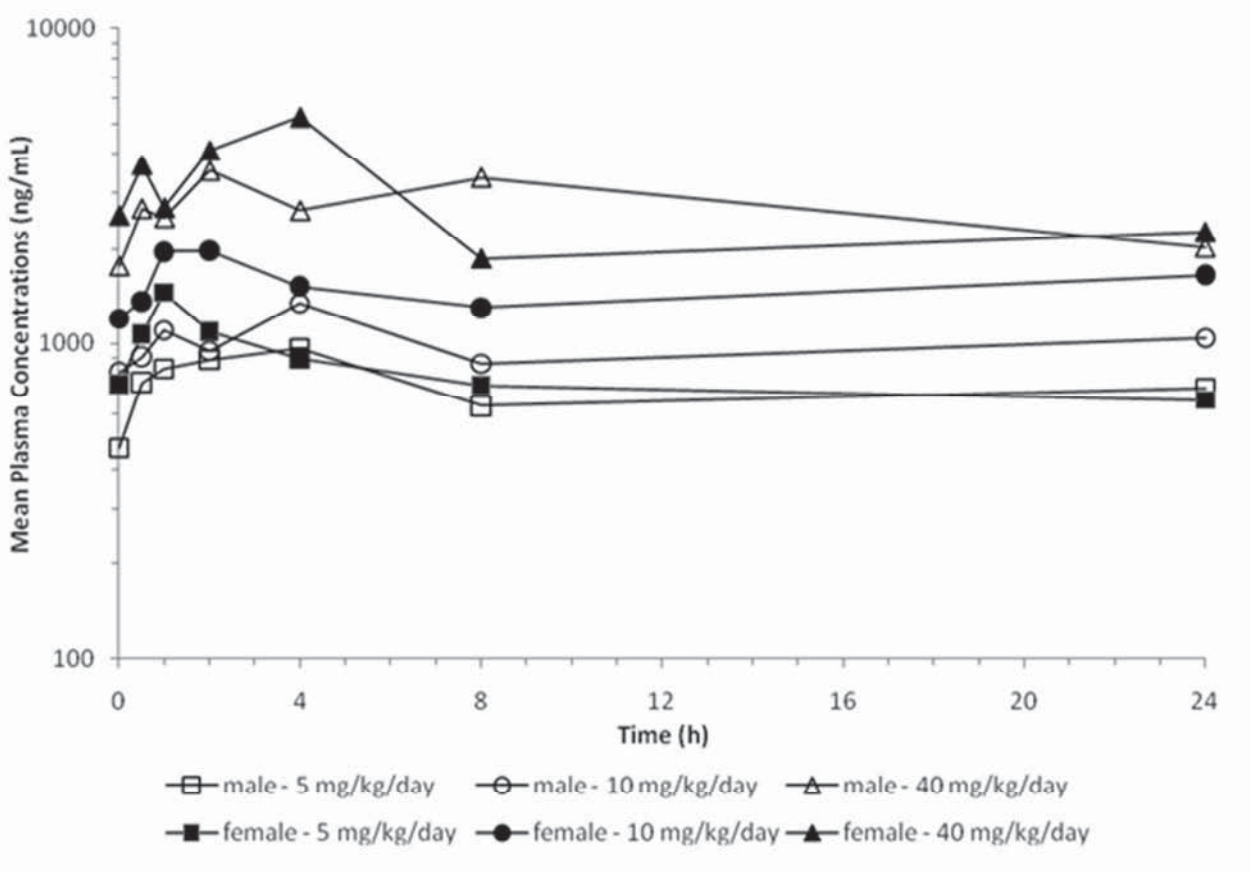
In the 1-month bridging study no clear difference was noted between exposure (AUC) obtained after administration of TMC278 base and TMC278. However, inter-individual variability was high especially after administration of TMC278.

Table 12. Pharmacokinetic Parameters of TMC278 After Single or Repeated Administration by Oral Gavage of TMC278 Base in Beagle Dogs

Study	Formulation	Dose Level (mg/kg/day)	Sampling Time (Day)	C_{max} (µg/mL) (CV in %)		AUC_{0-24h} (µg·h/mL) (CV in %)	
				Male	Female	Male	Female
12-month study in dogs (TMC278-NC107)	TMC278 base in PEG400/CA (10%)	5	1	0.70 (12.9)	0.75 (7.1)	11 (14.5)	9.7 (16.5)
			363	1.1 (12.8)	1.5 (42.4)	17 (13.5)	19 (32.2)
		10	1	0.90 (23.1)	1.2 (37)	15 (24.8)	15[b] (31.1)
			363	1.3[a]	2.2 (13.7)	24[a]	36[c] (22.1)
		40	1	2.4 (39.7)	2.5 (31.3)	37 (36.3)	41 (27.6)
			363	4.1 (55.6)	5.5[d] (57.8)	65 (63.1)	61[d] (52.1)

a n=2
b n=5
c 1 animal was sampled at Day 335
d n=3
CV: coefficients of variation

Figure 7. Plasma Concentrations of TMC278 in Beagle Dogs After Repeated Administration of TMC278 Base by Gavage Once Daily for 12 Months (Day 363)



3.3.2.5. RPV: Monkey, Oral

TMC278 formulated in aqueous HPMC (0.5%) was administered by oral gavage to female cynomolgus monkeys at 10 and 100 mg/kg/day for 7 days ((m2.6.5, Section 4.2.21, TMC278-NC249). TMC278 formulated in aqueous HPMC (1%)/Tween 20 (0.5%) was administered by oral gavage to female cynomolgus monkeys at 100 mg/kg BID and at 250 mg/kg BID (dosing interval of 8 hours) for 2 weeks ((m2.6.5, Section 4.2.22, TMC278-NC326). TMC278 formulated in aqueous HPMC (1%)/Tween 20 (0.5%) was also administered to immature female monkeys at 100 mg/kg BID and at 250 mg/kg BID (dosing interval of 8 hours) for 55 days (m2.6.5, Section 4.2.23, TMC278-NC248).

In all studies inter-individual variability was high with a CV for C_{max} and AUC higher than 50%. The absorption rate was moderate based on t_{max} values ranging between 2.5 and 8 hours after the first dose. After BID dosing, C_{max} after the first dose (C_{max1}) and the second dose (C_{max2}) were comparable. After repeated dose administration, plasma concentration-time profiles became rather flat. A dose-proportional increase in C_{max} and AUC was observed after single and repeated dosing for 2 weeks. After repeated administration for 2 weeks, exposure expressed as C_{max} and

AUC seemed to increase by 2-fold compared to Day 1. In the 55-day study in immature monkeys, C_{max} and AUC increased less than dose-proportionally after repeated dose administration (Table 13).

Table 13. Pharmacokinetic Parameters of TMC278 After Single or Repeated Administration by Oral Gavage of TMC278 in Female Cynomolgus Monkeys

Study	Formulation	Dose Level (mg/kg/day)	Sampling Time (Day)	C_{max1} (µg/mL) (CV in %)	C_{max2} (µg/mL) (CV in %)	AUC_{0-24h} (µg·h/mL) (CV in %)
2-week study in monkeys (TMC278-TiDP38-NC326)	TMC278 in HPMC (1%)/Tween 20 (0.5%)	100 BID	1	0.094 (72)	0.19 (62)	2.8 (64)
			14	0.29 (72)	0.21 (50)	4.4 (58)
		250 BID	1	0.25 (63)	0.38 (61)	5.9 (65)
			14	0.50 (42)	0.56 (52)	10 (42)
55-day study in immature monkeys (TMC278-TiDP38-NC248)	TMC278 in HPMC (1%)/Tween 20 (0.5%)	100 BID	55	0.14 (52)	0.18 (56)	2.7 (52)
		250 BID	55	0.31 (82)	0.31 (71)	4.6 (57)

a C_{max1} and C_{max2}: C_{max} after the first and second dose administration, respectively; dosing interval of 8 hours

3.3.3. TAF: Repeat-Dose In Vivo Studies

3.3.3.1. Mouse

GS-7340-02 was administered by oral gavage for up to 14 days to male and female Crl:CD1(ICR) mice at a dose of 100, 500, or 1000 mg/kg/day (m2.6.7, Section 6.3, TX-120-2006). Due to early death for animals given 500 or 1000 mg/kg/day, only the 100 mg/kg/day dose group was evaluated. GS-7340 at 100 mg/kg/day corresponded to a Day 14 C_{max} of 27.1 and 2.89 ng/mL for males and females, respectively; the AUC_{0-24} could not be calculated due to the lack of a distinct elimination phase. GS-7340 rapidly converted to its metabolite TFV. There were no significant differences in TFV pharmacokinetic profiles between males and females.

Following daily administration of GS-7340-02 to mice via oral gavage for at least 13 weeks at doses of 0, 10, 30, and 100 mg/kg/day, the pharmacokinetic parameters for TAF and TFV were determined (m2.6.7, Section 7.3.1, TX-120-2007). Blood samples were collected from up to 3 toxicokinetic animals/sex/group/timepoint on Day 1 and during Week 13 predose and at approximately 0.25, 0.5, 1, 4, 8, and 24 hours postdose. Exposure to TFV increased with the increase in GS-7340-02 dose from 10 to 100 mg/kg/day. The increases in C_{max} and AUC_{0-t} were generally greater than proportional between the 10 to 100 mg/kg/day dose levels. Gender-based

differences were less than 2-fold in TFV C_{max} and AUC_{0-t} values. No unexpected accumulation of TFV was observed after multiple dosing. Tenofovir alafenamide was rapidly and extensively converted to TFV after oral administration in mice.

3.3.3.2. Rat

The plasma pharmacokinetic profile of TFV was determined during the course of a 28 day oral gavage toxicity study in adult male and female albino (Sprague-Dawley) rats following daily administration of either 1.5, 6.25, 25, 100 or 400 mg/kg/day GS-7340-02 (m2.6.7, Section 7.3.2, R990182). A nonlinear pharmacokinetic response was observed for total plasma exposure versus dose for both sexes on both Day 1 and Day 28. A greater than linear increase in plasma exposure was observed as the dose was increased. There was no observed plasma accumulation (accumulation ratio 0.96 to 1.11) over the 28-day study for any dose group.

In a 26-week toxicology study, GS-7340-02 was administered once daily at doses of 0 (vehicle only), 5, 25 and 100 mg/kg/day by oral gavage and plasma pharmacokinetic parameters of TFV were determined on Day 1 and during Weeks 13 and 26 (m2.6.7, Section 7.3.3, TOX-120-001). No consistent differences in plasma pharmacokinetic parameters were found between male and female rats. Mean tenofovir C_{max} and AUC values increased dose proportionally over the dose range of 5 to 100 mg/kg/day. Mean tenofovir AUC obtained on Day 1 was slightly lower than that measured during Weeks 13 and 26, which suggested that there was a slight accumulation of tenofovir with repeat dosing.

3.3.3.3. Dog

Following daily oral administration of 8.29 mg/kg TAF for 7 days to male Beagle dogs, the plasma and liver pharmacokinetic profiles were determined on day 1 and 7 (m2.6.5, Section 4.3.1, AD-120-2033). Tenofovir alafenamide was rapidly absorbed and exhibited a short terminal half-life ($t_{½}$) of 0.3 hours in plasma on both Day 1 and 7. The rapid disappearance of TAF was accompanied by an increase in TFV. Tenofovir was the major metabolite detected in plasma achiving a maximal plasma concentration (C_{max}) of 1.47 and 2.12 µM on Day 1 and 7, respectively. The pharmacologically active diphosphate metabolite, TFV-DP, was efficiently formed in dog livers achieving concentrations of 242 and 153 µM at 4.0 and 24 hours postdose on Day 7, respectively.

The plasma PK of TAF and TFV and TFV levels in PBMCs were determined during the course of a 28-day oral gavage toxicity study in adult male and female beagle dogs following daily administration of either vehicle, 0.1, 0.3, 1.0, 3.0, or 10 mg/kg/day GS-7340-02 (m2.6.5, Section 4.3.2, D990175-PK). Repeat dosing at 10 mg/kg/day resulted in nonlinear pharmacokinetics between Days 1 and 28 with TAF median AUC values of 0.454 and 0.985 µg·h/mL, C_{max} values of 582 and 1280 ng/mL, and $t_{½}$ λ_z values of 18 and 23 minutes, respectively. The TFV C_{max} values appeared to be linear with increasing dose as well as repeat dosing. The TFV $t_{½}$ was estimated to be 37 h and substantial accumulation of TFV was observed after repeat dosing. The TFV levels in PBMCs were not linear with increasing dose; however, a linear correlation was observed between TFV levels in PBMCs and corresponding trough plasma concentrations. PBMC concentrations were approximately 100-fold higher than corresponding plasma concentrations.

In a 9-month toxicology study in dog, GS-7340-02 was administered once daily at doses of 0, 2, 6, and 18 mg/kg/day (m2.6.7, Section 7.3.5, TOX-120-002). The dose of 18 mg/kg/day was decreased to 12 mg/kg/day on Day 2 of Week 7 for males and Day 2 of Week 8 for females due to severe clinical signs and reduced body weight and food consumption. The concentrations of GS-7340 and tenofovir in plasma samples and total TFV in Week 39/40 PBMC samples were determined. GS-7340 was rapidly absorbed and converted to tenofovir following oral dose administration, with peak plasma concentrations of GS-7340 and tenofovir occurring at 0.5 and 1 hour postdose, respectively. GS-7340 was eliminated rapidly from the plasma with a terminal phase half-life of less than 1 hour. The median $t_{½}$ of tenofovir was estimated to be in the range of 25 to 31 hours on Day 1. The plasma pharmacokinetics of GS-7340 and tenofovir were comparable between male and female dogs after oral administration. Plasma C_{max} and AUC values for TAF increased more than proportionally over the dose range of 2 to 18/12 mg/kg/day. The plasma TFV C_{max} and AUC increased roughly dose proportional. There was some accumulation of tenofovir following repeat dosing (~3-fold). Tenofovir concentrations in PBMCs were measurable at 24-hour postdose for all dose groups. The median terminal phase half-life of total tenofovir in PBMCs was estimated to be 31 hours (similar to the tenofovir plasma estimate) from the recovery animals with PBMC concentrations measured up to 72 hours. Dose-normalized PBMC mean AUC values of total tenofovir increased more than dose proportionally during Week 39/40.

3.3.3.4. Monkey

Following daily oral administration of GS-7340-02 at 0, 3, and 30 mg/kg/day or TFV at 15 mg/kg/day for 28 days, pharmacokinetic profiles of TAF and/or TFV were determined on Day 1, Day 14 and Day 28 (m2.6.5, Section 4.3.3 and m2.6.7, Section 7.3.6, P2000114-PK). Concentrations of TFV in PBMCs were determined on Day 14 and Day 28. No significant differences in pharmacokinetic parameters were found between males and females. The pharmacokinetic parameters for TFV were dose linear on Day 1 but were greater than dose-linear on Day 28 after oral administration of GS-7340-02. There was no statistically significant accumulation of TFV following repeat dosing of either GS-7340-02 or TFV. The intracellular TFV concentrations in PBMCs were only determined from the 30 mg/kg/day GS-7340-02 dose group where 72.3 and 27.2 µg/mL were detected on Day 14 and Day 28, respectively.

3.4. FTC/RPV/TAF: Absorption

With respect to potential drug interactions within the combination that could affect absorption, FTC and RPV shows high passive permeability and is unlikely to be affected when administered with TAF. While TAF is an efflux substrate in the intestine, absorption is unlikely to be affected by FTC as intestinal efflux transport is not inhibited by FTC. Inhibition of P-gp by RPV observed in vitro is unlikely to be clinically relevant as RPV did not affect plasma exposure to P-gp substrates including digoxin and TAF in clinical drug interaction studies (m2.7.2, Sections 1.2.1 and 1.2.2, [TMC278IFD1001, GS-US-120-0117, and GS-US-120-1554]). Although formal nonclinical studies of the absorption kinetics of the FTC/RPV/TAF FDC have not been conducted, comprehensive clinical studies on the combination have been performed (m2.7.2, Section 1.2).

4. DISTRIBUTION

4.1. Plasma Protein Binding

4.1.1. FTC: Plasma Protein Binding

The binding of FTC to mouse, rabbit, monkey, and human plasma was determined over the concentration range 0.020 to 200 µg/mL by equilibrium dialysis at 37ºC (m2.6.5, Section 6.1.1, TBZZ/93/0025). The mean percentage bound for all species studied was ≤ 3.6%, with no indication of concentration dependence.

4.1.2. RPV: Plasma Protein Binding

The plasma protein binding of TMC278 was studied in vitro by equilibrium dialysis. Plasma samples from male and female CD-1 mice; male and female Sprague Dawley rats; female New Zealand white rabbits; male beagle dogs; and healthy male adult subjects were fortified with ^{3}H-TMC278 at concentrations ranging from 0.01 to 100 µg/mL (animals) and from 0.01 to 3.0 µg/mL (human) (m2.6.5, Section 6.2.1, TMC278-NC112). The distribution of TMC278 to various compartments of blood and the binding of TMC278 to purified human serum albumin and α_1-acid glycoprotein were also studied (m2.6.5, Section 6.2.1, TMC278-NC112). The distribution of [^{14}C]TMC278 in blood and the protein binding of [^{14}C]TMC278 in plasma were also investigated in samples from female guinea pigs at 2.5 and 8 µg/mL and from female monkeys at 2.5 and 5 µg/mL (m2.6.5, Section 6.2.2, TMC278-NC332). In addition, the protein binding of unlabeled TMC278 was determined in plasma samples from male and female CD-1 mice; male and female Sprague Dawley rats; male beagle dogs; and healthy male subjects at a concentration of 0.03, 0.3 or 1 µg/mL (animals) and 0.01, 0.03, 0.1, 0.3, 1, or 3 µg/mL (human) (TMC278-FK4217).

TMC278 was extensively bound to plasma proteins in all species and the plasma protein binding was found to be concentration independent. Plasma protein-binding values ranged between 99.08% and 99.97%. TMC278 was extensively bound to human albumin (99.5% at a physiological concentration of 4.3% and irrespective of the TMC278 concentration) and to a much lesser extent to α_1-acid glycoprotein (48.8% at a physiological concentration of 0.07% and a TMC278 concentration of 1 µg/mL). The rank order of blood to plasma concentration ratio in all species was monkey > dog > rat > man > guinea pig > rabbit > mouse and ranged from 0.96 to 0.58. In all species, irrespective of the concentration, a very limited percentage of TMC278 distributed to the plasma water compartment and the values ranged from 0.06% to 0.5%. In guinea pig, male and female rats, male dog, monkey, and man, the percentage of TMC278 distributed to plasma proteins ranged from 61.8% to 94.1%, and the percentage of TMC278 distributed to blood cells ranged from 5.8% to 37.6%. In mouse and rabbit, TMC278 distributed almost completely to plasma proteins (see Table 14).

The protein binding results of unlabeled TMC278 were in line with the ones obtained with radiolabeled compound at the same range of concentrations. No data were obtained at 0.01 and 0.03 µg/mL due to the fact that concentrations were below the limit of quantification.

Table 14. Plasma Protein Binding and Blood Distribution of TMC278 at 1 µg/mL (Various Species) or 2.5 µg/mL (Guinea pig and Monkey)

	Mouse		Rat		Guinea Pig	Rabbit	Dog	Monkey	Human
	M	F	M	F	F	F	M	F	M
Plasma protein binding (%)	99.93	99.94	99.84	99.86	99.87	99.97	99.35	99.14	99.67
Free drug (%)	0.07	0.06	0.16	0.14	0.13	0.03	0.66	0.86	0.33
Blood to plasma ratio	0.60	0.58	0.67	0.67	0.64	0.61	0.68	0.96	0.66
Distribution to									
Plasma water (%)	0.07	0.07	0.15	0.14	0.11	0.03	0.50	0.53	0.20
Plasma proteins (%)	100	> 100	93.0	94.1	84.7	> 100	73.4	61.8	78.1
Blood cells (%)	< 0	< 0	6.8	5.8	15.2	< 0	26.2	37.6	21.7

4.1.3. TAF and TFV: Plasma Protein Binding

Since TAF is highly unstable in rodent plasma due to high levels of plasma esterases expressed in some rodent species, the extent of TAF binding to plasma could only be determined in dog and human plasma in vitro (m2.6.5, Section 6.3.1, AD-120-2026). Protein binding of TAF was moderate in dog and human plasma with the percent unbound values of 48.0% and 46.8%, respectively. These in vitro values were higher than those observed in multiple human ex vivo studies with the mean percent unbound TAF ranging from 14% to 23% in all subjects (GS-US-120-0108 and GS-US-120-0114). Since the ex vivo results should be more clinically relevant, percent unbound TAF of 20% was used for the assessments for potential drug interactions (Section 7.3).

The protein binding of TFV has been determined in human plasma and serum using centrifugal ultrafiltration over the range of 0.01 to 25 µg/mL (m2.6.5, Section 6.3.2, P0504-00039.1). The percent unbound for TFV was 99.3 ± 3.3% in human plasma, and 92.8 ± 3.6% in human serum. Tenofovir therefore showed very low protein binding in either human plasma or serum.

4.2. Tissue Distribution Studies

4.2.1. FTC: Tissue Distribution Studies

4.2.1.1. FTC: Rat

To examine brain penetration of FTC, male Sprague-Dawley rats were administered FTC intraperitoneally at 10 mg/kg, and brain and plasma concentrations were determined. Brain/plasma ratios were low (3.4%–6.9%) and were unaffected by pretreatment with probenecid (60 mg/kg 15 minutes prior to treatment with FTC) {4570}.

Male Sprague-Dawley nonpigmented rats and Long Evans pigmented rats received a single 200 mg/kg oral dose containing approximately 135 μCi/kg of [^{14}C]FTC via gavage (m2.6.5, Section 5.1.1, TOX092). The distribution of radioactivity in the nonpigmented tissues of Long Evans pigmented rats was similar to that of Sprague-Dawley rats (56 tissues assessed). For both groups, the absorption of [^{14}C]FTC following oral administration was rapid, and the radioactivity was widely distributed among all of the examined tissues. The clearance of radioactivity from the plasma and tissues was also rapid with a $t_{½}$ of approximately 1 to 6 hours for blood and tissues, and 2.7–3.4 hours for plasma. In agreement with the previous study with unlabeled FTC, tissue:plasma ratios for radioactivity in CNS tissues were all < 0.1.

The pharmacokinetic parameters for [^{14}C]FTC derived radioactivity in eyes and skin were not markedly different for nonpigmented and pigmented rats, indicating that [^{14}C]FTC-associated radioactivity does not bind appreciably to melanin.

4.2.1.2. FTC: Monkey

To assess brain penetration of FTC, cynomolgus monkeys were dosed orally with 40, 200, and 1000 mg/kg of FTC and concentrations were determined in plasma and CSF. Concentrations of FTC in the CSF were dose- and concentration-independent and were 4% ± 0.7% of the corresponding levels in plasma {4251}.

Male cynomolgus monkey received an oral dose of 200 mg/kg FTC containing 42.9 μCi/kg [^{14}C]FTC. The tissue distribution of radioactivity after oral administration of [^{14}C]FTC in the cynomolgus monkey is described in detail in Section 5.3.1 (m2.6.5, Section 10.1.2, TOX063). Radioactivity was widely distributed to all tissues by 1 hour postdose. Plasma concentrations of radioactivity declined in parallel of those of parent FTC. Concentrations of radioactivity in tissues were similar to those in plasma except for the GI. The mean whole blood to plasma ratio was 0.87.

4.2.2. RPV: Tissue Distribution Studies

4.2.2.1. RPV: Rat

The tissue distribution of TMC278 and its metabolites was studied in male pigmented Long Evans rats and pregnant female Sprague Dawley rats by QWBA, following a single oral dose of [^{14}C]TMC278 base in PEG400/CA (10%) at 40 mg/kg (m2.6.5, Section 5.2, TMC278-NC108 and TMC278-NC109). The tissue distribution of unchanged TMC278 was assessed in a limited set of tissues obtained in a pharmacokinetic study in male Sprague Dawley rats given a single oral dose of 40 mg/kg (PEG400/CA (10%) (m2.6.5, Section 3.2.3, TMC278-FK4195).

In pigmented rats, highest total radioactivity levels were observed at 4 hours postdose in nonpigmented and most pigmented tissues, indicating a rapid distribution of [^{14}C]TMC278 related radioactivity. Only in the pigmented parts of the eye and the uveal tract, the highest concentration of radioactivity was measured at 24 hours after dosing.

In most non-pigmented tissues, radioactivity levels were only quantifiable by radioluminography (RLG) until 4 hours after dosing. The highest concentration of radioactivity was measured in the liver and the exposure ($AUC_{0–4h}$) was 12-fold higher than the $AUC_{0–4h}$ observed in blood (Figure 8). In the adrenal gland, brown fat and kidney $AUC_{0–4h}$ values were about 4- to 5-fold those in blood. In pancreas and white fat, the $AUC_{0–4h}$ values were almost 3-fold that in blood. In spleen, based on C_{4h}, the radioactivity level was almost 3-fold that in blood. Tissue to blood $AUC_{0–4h}$ ratios in lung, heart, white skin, and thyroid were about 2. $AUC_{0–4h}$ values in prostate gland, bone marrow, muscle, testis and brain were similar or a bit lower than those in blood.

In pigmented tissues, the radioactivity decreased more slowly than in the other tissues and was still quantifiable by RLG 14 days postdose. Tissue to blood $AUC_{0–336h}$ ratios were 146 (uveal tract), 18 (brain meninges) and 15 (pigmented skin). Although levels in pigmented tissues at 14 days postdose still represented about 20% of corresponding peak levels, radioactivity levels decreased from 4 or 24 hours onwards. Therefore no undue retention of [^{14}C]TMC278 derived material is expected.

The QWBA study in non-pigmented pregnant rats showed a similar tissue distribution profile as the one observed in the male rat (Figure 9). In most tissues, highest radioactivity levels were observed at 4 hours postdose, indicating rapid distribution into the tissues. Only in uterine epithelium and to a lesser extent in adrenal gland, radioactivity concentrations declined more slowly than in blood from 8 to 24 hours.

Some specific reproductive tissues were evaluated. The AUC_{0-8h} values of uterine epithelium, mammary gland, and ovary were 4-, 3-, and 2-fold higher than the corresponding blood AUC_{0-8h} values, respectively. The $AUC_{0–8h}$ values in uterus, placenta, and vagina were similar to slightly lower than those in blood. The $AUC_{0–8h}$ in whole fetus was 0.64-fold that in maternal blood, suggesting that the placenta presents a partial barrier for TMC278 and/or its metabolites.

In a pharmacokinetic study after administration of non-radiolabeled TMC278 base in male Sprague Dawley rats, plasma, and tissue samples from the adrenal glands, brain, liver, and muscle were collected. Maximum tissue concentrations were observed within 20 to 60 minutes after administration. Tissue levels declined in parallel with plasma concentrations. Highest tissue concentrations were observed in the liver and the adrenal gland reaching tissue to plasma AUC_{0-24h} ratios of 3.4 and 2.7, respectively. In brain and muscle, the tissue to plasma ratios were 0.49 and 0.45, respectively. These results were similar to the data obtained with radiolabeled material, showing high distribution to the liver and adrenal glands and lower distribution to the muscles and brain.

Figure 8. **Tissue to Blood $AUC_{0–4h}$ Ratios of Total Radioactivity, as Determined by Radioluminography of Whole-Body Sections of Tissues in the Male Pigmented Long Evans Rats After a Single Oral Administration of ^{14}C-TMC278 base at 40 mg/kg**

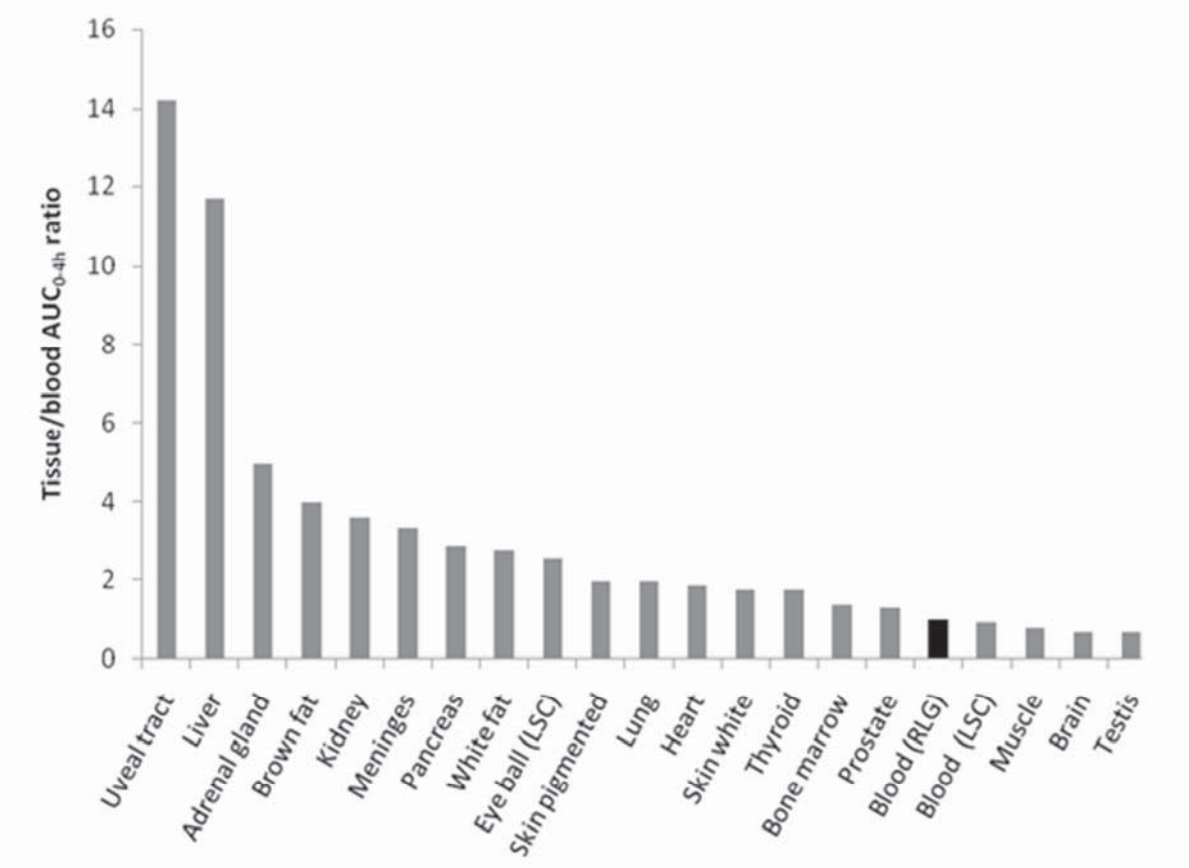
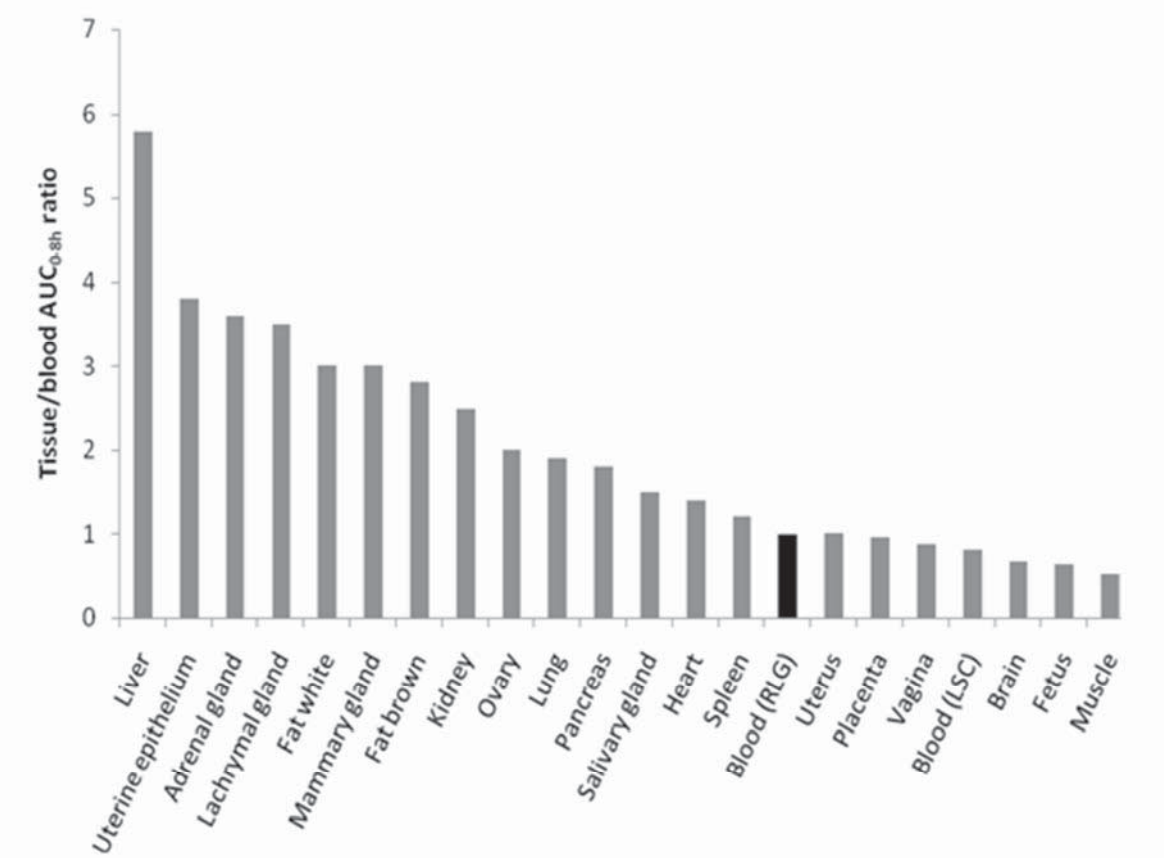


Figure 9. Tissue to Blood $AUC_{0–8h}$ Ratios of Total Radioactivity, as Determined by Radioluminography of Whole-Body Sections of Tissues in the Pregnant Female Sprague Dawley Rats After a Single Oral Administration of ^{14}C-TMC278 base at 40 mg/kg



4.2.2.2. RPV: Dog

Tissue distribution assessments were conducted on some tissues collected in association with repeat dose administration in toxicology studies. Plasma and adrenal glands were obtained from dogs (3 animals/sex/group) following a 1-month toxicity study (m2.6.5, Section 4.2.17, TMC278-TOX5650). Plasma, adrenal gland, and liver were also collected from dogs (3 animals/sex/group) following a 6-month study (m2.6.5, Section 4.2.19, TMC278-NC115). In both studies, animals received TMC278 base (PEG400/CA (10%) oral doses of 5, 10, and 40 mg/kg/day. Plasma and tissues were collected at autopsy on Day 28/29 and Day 56 (recovery period) in the 1-month study or on Day 93 and Day 184/185 in the 6-month study.

In both studies, the adrenal gland concentrations were higher than the corresponding plasma concentrations with mean tissue to plasma concentration ratios ranging from 1.3 to 8.1. After 1 month of recovery, however, the adrenal gland levels as well as the plasma levels decreased below the limit of quantification. In the liver, TMC278 concentrations were also higher than in plasma, with tissues to plasma concentration ratios ranging from 7.7 to 13. In the 6-month study, adrenal, and liver tissue to plasma ratios were similar between Day 93 and Day 184/185 of the study.

4.2.3. TAF: Tissue Distribution Studies

4.2.3.1. TAF: Mouse

After oral dosing of 100 mg/kg [^{14}C]TAF to male CD-1 mice, [^{14}C]TAF-derived radioactivity was widely distributed to most of the tissues by the first collection time point (0.5 hours postdose) (m2.6.5, Section 5.3.1, AD-120-2011). Most tissues reached maximum concentration by 1 hour postdose. The tissues showing the highest maximum concentrations of radioactivity, excluding GI tract, included liver, gall bladder, urinary bladder, diaphragm, kidney cortex, kidneys, and kidney medulla. The tissues with the lowest C_{max} values were testis, brain cerebrum, fat (abdominal), spinal cord, and brain medulla.

In male C57 Black mice, [^{14}C]TAF-derived radioactivity was widely distributed to most of the tissues by the first collection time point (0.5 hours postdose), similar to CD-1 mice. Most tissues reached maximum concentration by 0.5 hours postdose. The tissues showing the highest maximum concentrations of radioactivity, excluding the GI tract, included liver, gall bladder, urinary bladder, kidney cortex, kidneys, kidney medulla, and diaphragm. The tissues with the lowest C_{max} values were testis, spinal cord, brain cerebrum, brain medulla, and brain cerebellum.

Low levels of radioactivity were detected in brain in mice suggesting [^{14}C]TAF-derived radioactivity poorly crossed the blood:brain barrier. Low levels of radioactivity were also measured in testis in mice, suggesting [^{14}C]TAF-derived radioactivity poorly crossed the blood:testis barrier.

More persistent exposures in eye lens, eye uveal tract, and eyes were observed in CD57 black mice compared to CD-1 mice. However, no difference in distribution between pigmented and nonpigmented skin was observed illustrating that ^{14}C-TAF-related radioactivity was not selectively associated with melanin-containing tissues. Comparative quantification data for selected time points are provided in Table 15.

Table 15. Comparative Tissue Concentrations of Radioactivity in Male CD-1 and C57 Black Mice After Oral Administration of [^{14}C]TAF (n = 1 per time point)

Organ	Radioactivity (µg equivalents ^{14}C-TAF/g tissue)									
Time point	0. 5 h		1 h		3 h		12 h		24 h	
Rat Strain	CD-1	C57 Black	CD-1	C57 Black	CD-1	C57 Black	CD-1	C57 Black	CD-1	C57 Black
Adrenal gland(s)	9.92	34[a]	27.5	26.4	9.45	20.4	3.01	7.75[a]	3.6	10.4[a]
Bile	60.3	198	127	136	115	150	65.9	40.3	12.9	26.6
Blood	14.6	16.7	14.5	9.27	5.28	7.91	4.38	4.17	6.1	3.78
Bone	1.3	11.2	2.42	4.49	1.23	4.9	1.01	2.43	0.525	1.77
Bone marrow	4.73	24.3	6.61	19	2.03	16.1	2.04	6.44	2.12	6.47
Brain cerebellum	0.569	0.611	1.94	0.59	0.395	BLQ	BLQ	ND	BLQ	ND
Brain cerebrum	0.768	1.24	1.43	0.661	BLQ	BLQ	0.379	ND	BLQ	ND
Brain medulla	0.42	0.886	0.833	BLQ	BLQ	BLQ	BLQ	ND	BLQ	ND
Brain olfactory lobe	1.12	3.79	2.89	1.73	0.464	1.64	0.461	0.688	BLQ	0.707
Cecum	3.44	25.4	6.96	95.4	90.5	ND[b]	19.1	50.5	8.31	10.5
Diaphragm	40.6	60.3	167	103	46.6	114	26.6	27.5	28.3	25.9
Epididymis	3.6	10.8[a]	22.3	4.19	1.31	4.99	0.54	2.12	0.864	2.15[a]
Esophagus	38.4	76.1	90.8	81.3	57.3	48.9	20.1	27.9	8.66	8.54
Exorbital lacrimal gland	3.6	18.1	5.46	12	1.4	11.8	1.25	4.91	1.29	3.53
Eye lens	0.967	4.31	2.98	1.87	0.508	1.11	BLQ	0.527	BLQ	BLQ
Eye uveal tract	4.18	13.4	3.69	12.2	0.754	11.6	0.947	4.74	0.779	6.14
Eye(s)	1.88	5	2.66	2.92	0.539	2.44	0.428	1.04	0.363	1.06

Organ	Radioactivity (µg equivalents ^{14}C-TAF/g tissue)									
Time point	**0. 5 h**		**1 h**		**3 h**		**12 h**		**24 h**	
Rat Strain	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**
Fat (abdominal)	1.25	5.46	1.17	6.82	0.968	2.14	0.512	4.18	0.787	1.7
Fat (brown)	3.07	19.2	5.59	18.7	2.53	14.5	2.06	10.8	3.03	8.66
Gall bladder	335	163	216	379	108	275	68.1	94	37.1	39.8
Harderian gland	4.24	18.5	6.5	14.6	1.53	13.6	2.17	6.2	1.39	4.58
Intra-orbital lacrimal gland	7.63	NR	3.58	NR	1.9	NR	2.28	NR	1.5	3.76
Kidney cortex	92.3	137	89	125	74	104	30	58	23.1	34.5
Kidney medulla	65	125	70	94.7	54.8	80	18.1	40.2	12.8	19.4
Kidney(s)	84.8	132	86.1	107	68.9	89.6	25.9	47.5	19.9	29.5
Large intestine	6.68	25.7	8.09	17.3	7.73	32.3	22.5	74.2	6.77	35
Liver	282	488	447	490	290	385	197	282	164	118
Lung(s)	13.2	32.3	23.9	32.5	4	26.5	10	18.1	11.5	13.5
Lymph node(s)	5.19	17.9	7.75	14.6	1.68	12.6	1.76	8.02	2.28	10.9
Muscle	1.68	8.78	2.1	4.14	0.466	3.19	0.768	2.01	0.579	3.9
Myocardium	7.57	23.2	11.7	13.5	2.38	13.6	4.6	8.47	4.62	9.51
Nasal turbinates	2.72	10.6	6.44	2.98	1.29	3.55	0.983	2.39	1.19	1.92
Pancreas	6.13	34.9	14.5	26.9	2.95	31.5	3.54	15.4	2.88	13.9
Pituitary gland	2.91	17.4	7.44	8.44	1.28	6.59	2.51	0.85	1.3	ND
Preputial gland	1.9	11.5	2.58	6.39	0.635	6.13	0.708	2.24	0.503	1.97
Prostate gland	5.5	ND[c]	2.88	8.63	2.15	4.84	3.06	8.62	2.59	ND

Organ	Radioactivity (µg equivalents ^{14}C-TAF/g tissue)									
Time point	**0. 5 h**		**1 h**		**3 h**		**12 h**		**24 h**	
Rat Strain	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**
Salivary gland(s)	5.39	36.2	11.5	24.2	1.88	27.2	2.42	9.91	2.16	12.2
Seminal vesicle(s)	1.79	5.54	3.41	3.53	0.957	2.47	0.852	2.15	0.62	0.825
Skin (nonpigmented)	8.52	NA	5.58	NA	1.51	NA	0.674	NA	0.715	NA
Skin (pigmented)	NA	11.6	NA	5.93	NA	3.35	NA	1.18	NA	1.34
Small intestine	8.77	26.9	74.5	56.1	88.1	28	14.1	14.1	4.04	11.3
Spinal cord	0.757	1.95	1.21	0.742	BLQ	BLQ	BLQ	BLQ	BLQ	ND
Spleen	6.1	35.3	12.9	29.6	3.85	29.1	5.46	16.6	6.27	12.2
Stomach	77.6	60.5	39.3	70.6	26.6	29.2	5.46	7.9	5.94	6.73
Stomach mucosa	96.9	ND	46.9	ND	19.8	ND	5.28	ND	3.66	ND
Stomach wall	48.6	ND	24.5	ND	26.6	ND	7.69	ND	22.7	ND
Testis(es)	1.16	2.64	1.49	1.76	0.774	1.07	BLQ	0.512	0.319	0.752
Thymus	2.51	12.4	6.14	8.19	0.914	7.19	0.903	3.44	1.19	3.41
Thyroid	7.12	40.7	12.1	32.7	2.83	29	1.2	NR	4.6	10.9
Urinary bladder	ND[c]	ND[c]	174	138	85.6	49.1	5.8	12	10.3	6.29
Urine	1790	1170	413	626	200	128	36.8	135	24.7	18.4

BLQ = below the limit of quantitation [<311 (CD-1) or <490 (C57 Black) µg equivalents ^{14}C-TAF/g]; h = hours; NA = not applicable; ND = not detectable (sample shape not discernable from background or surrounding tissue); NR = not represented (tissue not present in section).

a Tissue appeared to be fat-soaked.
b Not detectable due to flare from cecum contents.
c Not detectable due to flare from urine.

4.2.3.2. TAF: Rat

After oral dosing of 5 mg/kg [^{14}C]TAF to male Sprague-Dawley or Long Evans rats, [^{14}C]TAF-derived radioactivity was widely distributed to most tissues by the first collection time point (0.25 hours postdose) (m2.6.5, Section 5.3.2, AD-120-2020). Representative autoradiographic images from animals terminated at 0.25 hours postdose are provided in Figure 10 (nonpigmented) and Figure 11 (pigmented). Comparative quantification data for selected time points are provided in Table 16. A full listing of tissue concentrations of radioactivity is provided in tabular form in m2.6.5 (Section 5.3.2 for albino animals and pigmented animals). In both Sprague-Dawley and Long Evans rats, most tissues reached maximum concentrations by the first collection time point. The tissues showing the highest maximum concentrations of radioactivity included kidney cortex, kidney(s), kidney medulla, and liver.

The tissues with the lowest C_{max} values were brain olfactory lobe, seminal vesicle(s), eye vitreous humor, thymus, eyes, testis(es), and harderian gland for Sprague-Dawley rats and bone, brain olfactory lobe, seminal vesicle(s), fat (abdominal), muscle, eye vitreous humor, and eye(s) for Long Evans rats. Transient exposure to low levels of ^{14}C-TAF-related radioactivity was observed in the eyes of rats decreasing to undetectable levels at 8 hours postdose. No difference in distribution was observed between Sprague-Dawley and Long Evans rats, including in the skin and eyes, suggesting no binding to melanin.

Table 16. Comparative Tissue Concentrations of Radioactivity in Male Sprague-Dawley and Long Evans Rats After Oral Administration of [^{14}C]TAF (n = 1 per time point)

Organ	Radioactivity (ng equivalents ^{14}C-TAF/g tissue)									
Time point	0.25 h		1 h		4 h		12 h		24 h	
Rat Strain	SD	LE	SD	LE	SD	LE	SD	LE	SD	LE
Adrenal gland(s)	181[a]	353[a]	129[a]	115	BLQ[a]	48.5[a]	ND	ND	ND	ND
Arterial wall	817	1350	299	270	118	90.4	ND	ND	ND	ND
Bile	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blood	1070	1260	334	221	138	116	83.1	117	ND	ND
Bone	BLQ	50.4	ND	BLQ	ND	ND	ND	ND	ND	ND
Bone marrow	233	311	125	84.9	72.6	BLQ	BLQ	BLQ	ND	ND
Brain cerebellum	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain cerebrum	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain medulla	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain olfactory lobe	45.7	57.2	BLQ	51.0	ND	ND	ND	ND	ND	ND
Bulbo-urethral gland	396	831	177	209	236	ND	ND	ND	ND	ND
Cecum	218	603	132	118	541	889	NR	362	323	494
Diaphragm	210	353	145	124	52.9	55.8	ND	ND	ND	ND
Epididymis	249[a]	516	101[a]	79.3	BLQ	BLQ	ND	ND	ND	ND
Esophagus	341	923	222	218	187	186	58.9	65.8	ND	ND
Exorbital lacrimal gland	234	353	101	56.1	51.2	BLQ	ND	ND	ND	ND
Eye lens	BLQ	BLQ	ND	BLQ	ND	ND	ND	ND	ND	ND
Eye uveal tract	409	555	187	89.5	78.4	59.4	ND	ND	ND	ND

Organ	Radioactivity (ng equivalents ^{14}C-TAF/g tissue)									
Time point	**0.25 h**		**1 h**		**4 h**		**12 h**		**24 h**	
Rat Strain	**SD**	**LE**	**SD**	**LE**	**SD**	**LE**	**SD**	**LE**	**SD**	**LE**
Eye vitreous humor	84.9	139	89.3	BLQ	BLQ	BLQ	ND	ND	ND	ND
Eye(s)	86.1	150	92.7	BLQ	BLQ	BLQ	ND	ND	ND	ND
Fat (abdominal)	BLQ	70.0	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Fat (brown)	200	232	97.1	BLQ	48.2	ND	55.7	ND	ND	ND
Harderian gland	98.6	209	52.4	BLQ	BLQ	ND	ND	ND	ND	ND
Intra-orbital lacrimal gland	250	402	118	53.4	BLQ	49.1	ND	ND	ND	ND
Kidney cortex	10700	8000	12400	8890	11800	6980	8300	5150	2010	2440
Kidney medulla	8240	6900	5040	3670	2800	655	1010	757	317	367
Kidney(s)	9520	7750	8710	7570	8250	5160	4590	3000	1380	1310
Large intestine	364	548	140	91.5	55.8	474	ND	133	ND	132
Liver	6730	10300	6730	7800	4010	7710	3570	5610	1090	1380
Lung(s)	592	854	211	145	81.1	67.2	ND	66.3	ND	BLQ
Lymph node(s)	318[a]	422[a]	ND	94.9[a]	ND	ND	ND	ND	ND	ND
Muscle	101	115	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Myocardium	361	512	136	46.6	58.6	BLQ	ND	BLQ	ND	ND
Nasal turbinates	123	201	83.8	71.9	52.8	BLQ	ND	ND	ND	ND
Pancreas	215	274	120	82.1	68.9	53.5	52.0	ND	ND	ND
Pituitary gland	335	434	116	53.1	50.5	ND	76.2	ND	ND	ND
Preputial gland	140[a]	247	60.1[a]	67.9	BLQ	ND	ND	ND	ND	ND

Organ	Radioactivity (ng equivalents ^{14}C-TAF/g tissue)									
Time point	**0.25 h**		**1 h**		**4 h**		**12 h**		**24 h**	
Rat Strain	**SD**	**LE**	**SD**	**LE**	**SD**	**LE**	**SD**	**LE**	**SD**	**LE**
Prostate gland	134	247	88.6	BLQ	117	49.9	BLQ	ND	ND	ND
Salivary gland(s)	292	368	119	85.3	BLQ	BLQ	ND	BLQ	ND	ND
Seminal vesicle(s)	62.7	67.7	ND	BLQ	ND	ND	ND	ND	ND	ND
Skin (nonpigmented)	393	526	123	71.8	BLQ	BLQ	ND	ND	ND	ND
Skin (pigmented)	NA	623	NA	78.9	NA	BLQ	NA	ND	NA	ND
Small intestine	479	530	500	376	364	122	86.5	220	171	98.6
Spinal cord	BLQ	BLQ	ND	BLQ	ND	ND	ND	ND	ND	ND
Spleen	147	231	105	83.1	59.0	58.3	62.8	60.7	BLQ	BLQ
Stomach	475	682	126	113	66.6	71.4	ND	159	ND	BLQ
Testis(es)	98.2	157	50.9	BLQ	BLQ	BLQ	ND	BLQ	ND	ND
Thymus	91.2	181	68.1	BLQ	BLQ	BLQ	ND	ND	ND	ND
Thyroid	324	412	147	59.1	63.6	ND	ND	ND	ND	ND
Tooth pulp	646	793	228	194	84.4	78.7	ND	57.0	ND	NR
Urinary bladder	925	352[a]	205	155	ND[b]	125	1050	45.9	BLQ	48.3
Urine	112000	50200	34400	61000	51100	2280	14500	1140	988	1500

BLQ = below the limit of quantitation (<45.6 ng equivalents ^{14}C-GS-7340/g); h = hours; NA = not applicable; ND = not detectable (sample shape not discernable from background or surrounding tissue).

a Tissue appeared to be fat-soaked.

b Not detectable due to flare from urine.

Figure 10. Annotated Whole-Body Autoradiograph for a Male Sprague-Dawley Rat 0.25 hours after a Single Oral Administration of ^{14}C-GS-7340 at 5 mg/kg

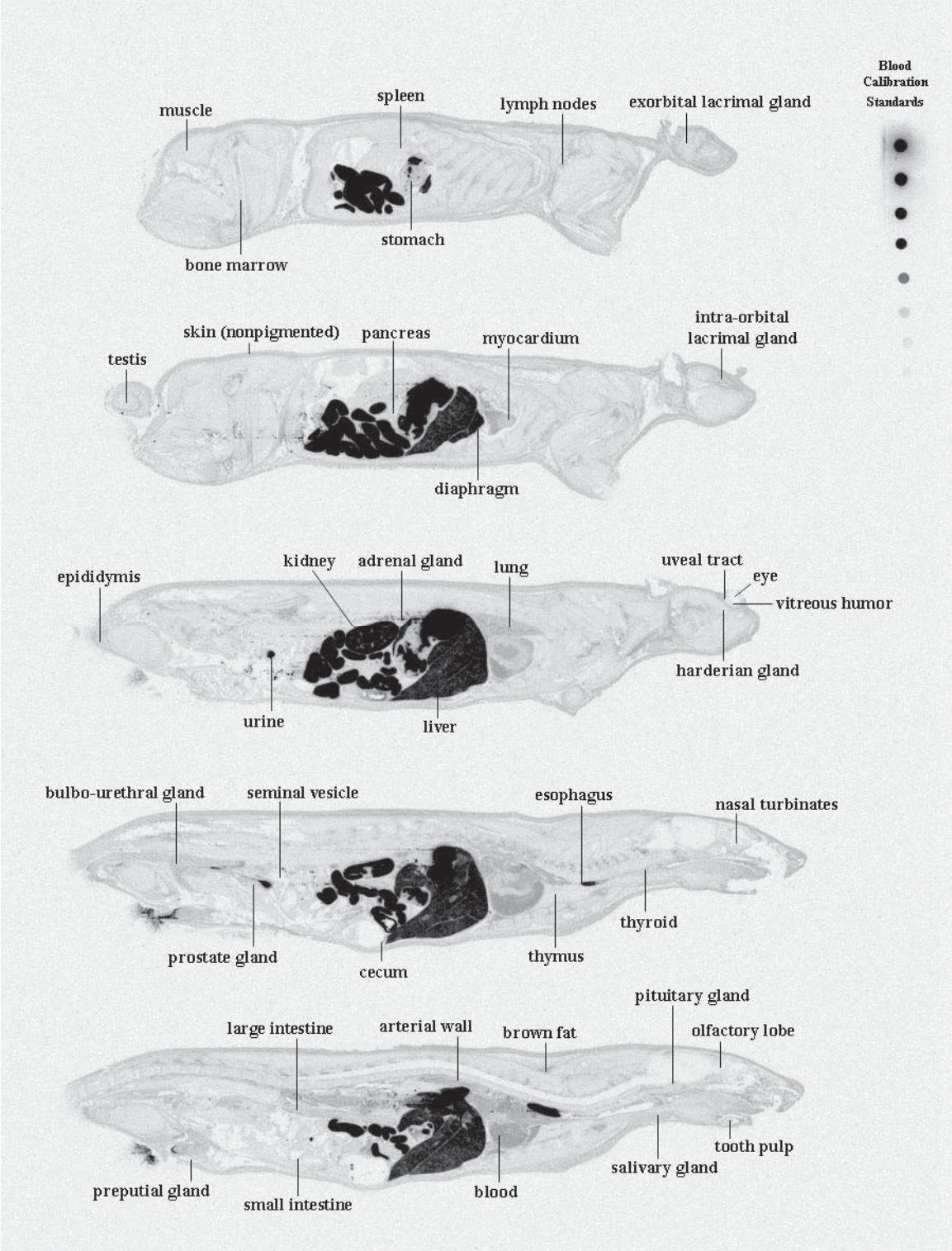
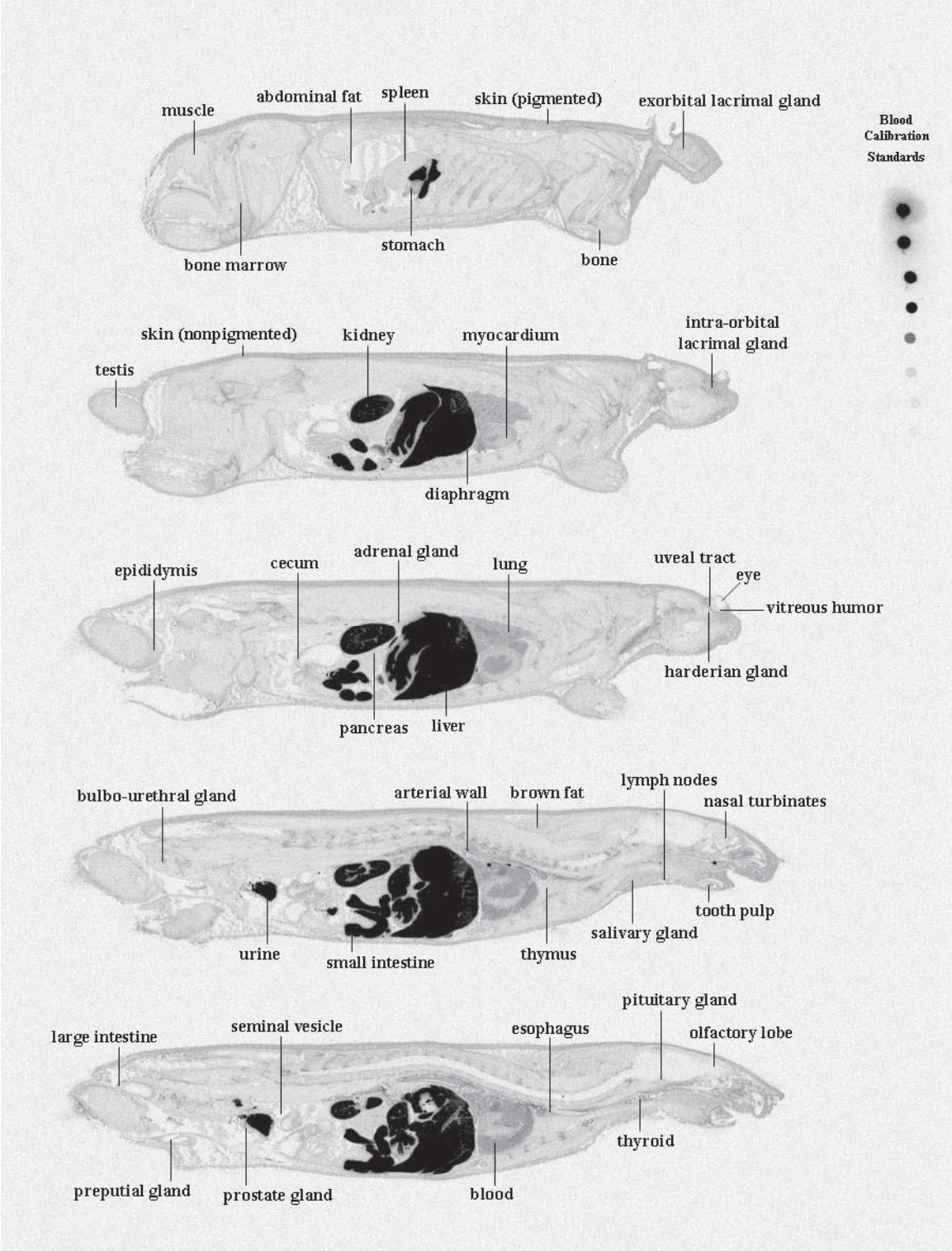


Figure 11. Annotated Whole-body Autoradiograph for a Male Long Evans Rat 0.25 hours after a Single Oral Administration of ^{14}C-GS-7340 at 5 mg/kg



4.2.3.3. TAF: Dog

The absorption and distribution of [^{14}C]TAF were determined following multiple 15-mg/kg oral doses of GS-7340 and a single oral dose of [^{14}C]TAF to male dogs at 15-mg/kg or at 18.1 mg/kg (m2.6.5, Section 5.3.3, AD-120-2009 and m2.6.5, Section 5.3.4, D990173-BP). Radioactivity was widely distributed but not detected in brain, eye, and cerebrospinal fluid. High concentrations were observed in kidney, liver, GI tract, spleen, lymph nodes and PBMCs. Following multiple or single oral administrations of TAF or [^{14}C]TAF to male dogs, the highest concentrations of radioactivity were observed in the liver and kidney through 24 hours postdose. Concentrations of radioactivity in tissues after multiple doses of unlabeled GS-7340 followed by a single dose of [^{14}C]TAF were higher compared to tissues from animals dosed with a single dose of [^{14}C]TAF only.

4.3. Studies in Pregnant or Nursing Animals

4.3.1. FTC

4.3.1.1. FTC: Pregnant Mice

Following oral administration of FTC to pregnant mouse dams, the exposure of murine fetuses to FTC was examined (m2.6.5, Section 7.1.1, TOX103 and Addendum report). Emtricitabine suspended in the vehicle, 0.5% methylcellulose (aqueous), was administered orally by gavage to Crl:CDR®-1(TCR) BR mice twice daily (approximately 6 hours between doses) from gestation Days 6 to 14 at a dose of 1000 mg/kg/day administered at a dose volume of 5 mL/kg/dose. On gestation Day 15, following administration of 500 mg/kg of FTC twice daily for approximately 10 days, pregnant mice and their viable fetuses had measurable concentrations of FTC 1 hour following administration of the first 500 mg/kg dose. The mean plasma concentration in the pregnant mice was 137.1 ± 28.0 µg/mL. The mean concentration of FTC in pooled fetal homogenate was 55.7 ± 10.4 µg/g. The mean fetal/maternal concentration ratio was 0.41 ± 0.04.

4.3.1.2. FTC: Pregnant Rabbits

In an oral GLP embryo-fetal toxicity study, pregnant New Zealand white rabbits (20/dose) were given FTC at 0, 100, 300, and 1,000 mg/kg/day in 0.5% aqueous methylcellulose as equal divided doses 6 hours apart on gestation Days 7 to 19. The does were necropsied on gestation Day 19 (m2.6.5, Section 7.1.2, TOX038 and Addendum report). Additional pregnant rabbits were dosed in parallel to provide plasma for systemic exposure assessment on gestation Day 19. They were killed at 1 hour postdose on Day 20 to provide maternal/fetal blood samples to confirm fetal exposures.

Emtricitabine was rapidly absorbed in dams with C_{max} occurring generally within 1 hour postdose. Systemic exposure to FTC (AUC and C_{max}) increased linearly with dose from 100 to 1000 mg/kg/day in both dams and fetuses. On gestation Day 19, AUC_{0-24} in dams was 87, 315, and 1258 µg·h/mL at 100, 300, and 1000 mg/kg/day, respectively. Plasma elimination $t_{½}$ was 3 to 4 hours at all dose levels. Fetal/maternal exposure ratios, as determined by analysis of umbilical cord blood, were around 0.4 to 0.5 at 1 hour after dosing (at t_{max}) for all dose levels. Emtricitabine was therefore readily transferred across the placenta (TOX038 and Addendum report).

4.3.2. RPV

4.3.2.1. RPV: Pregnant Rats

The placental transfer of TMC278 was studied in pregnant Sprague Dawley rats, by QWBA, after a single oral dose (gavage) of [^{14}C]TMC278 base in PEG400/CA (10%) at 40 mg/kg (m2.6.5, Section 5.2.2, TMC278-NC109). The $AUC_{0–8h}$ values in the placenta and in whole fetuses were 0.95- and 0.64-fold the $AUC_{0–8h}$ value of maternal blood, respectively. This suggests that the placenta is only a partial barrier for TMC278 and its metabolites.

4.3.3. TAF

4.3.3.1. TAF: Pregnant Rats

Tenofovir concentrations were determined in plasma from pregnant female rats dosed with GS-7340-02 by oral gavage for at least 12 days (Gestation Days [GD] 6 to 17) at 5, 100, and 200 mg/kg as an oral range-finding study (m2.6.7, Section 11.3, TX-120-2001) or at 25, 100, and 250 mg/kg as an embryo-fetal development study (m2.6.7, Section 13.5, TX-120-2002). Blood samples were collected on GD 6 and 17. Following oral gavage of GS-7340, concentrations of TFV readily appeared in plasma. Exposure to TFV increased with the increase in dose. The increases in C_{max} and AUC_{0-t} were generally greater than dose proportional between the 5 to 200 mg/kg/day dose levels in the range-finding study. In the embryo-fetal development study, C_{max} and AUC_{0-t} were inconsistently proportional between the 25 to 250 mg/kg/day dose levels. While accumulation of TFV was observed after multiple dosing of GS-7340-02 in pregnant rats in the range-finding study, no accumulation of TAF and TFV was observed in the embryo-fetal development study.

4.3.3.2. TAF: Pregnant Rabbits

The toxicokinetics of TAF and TFV were determined in plasma from pregnant female rabbits following administration of GS-7340-02 via oral gavage once daily on Gestation Days (GD) 7 through 20. GS-7340-02 was administered at dose levels of 5, 25, 50, and 100 mg/kg/day as an oral range-finding study (m2.6.7, Section 11.3, TX-120-2004) or at 10, 30, and 100 mg/kg/day as an embryo-fetal development study (m2.6.7, Section 13.6, TX-120-2005). Blood samples were collected from all animals on GD 7 and 20 predose and approximately 30 minutes and 2, 4, 8, and 24 hours postdose. Tenofovir alafenamide increased with the increase in GS-7340-02 dose level among all groups. The increases in C_{max} and AUC_{0-t} were greater than dose proportional in all dose levels in both studies. Exposure to TFV increased with the increase in GS-7340-02 dose level and the increases in C_{max} and AUC_{0-t} were approximately proportional in all dose levels. No unexpected accumulation of TFV was observed after repeat dosing of GS-7340-02 in rabbits. GS-7340 was extensively converted to TFV in rabbits following oral administration of GS-7340-02.

4.3.3.3. TFV: Pregnant Monkeys

Placental transfer of TFV following subcutaneous administration to a pregnant rhesus monkey was determined (m2.6.5, Section 7.3.2.1, 96-DDM-1278-005). One rhesus monkey received daily subcutaneous injection of 30 mg/kg/day TFV, beginning at Day 111 of gestation. Maternal and fetal blood samples were drawn at Days 115, 127, 134, 140, and 151 of gestation. Placental transfer of TFV appeared to be significant with a fetal/maternal serum concentration ratio of 0.17 ± 0.07 (mean ± SD) at approximately 30 minutes postdose.

4.3.3.4. TFV: Lactating Monkeys

The pharmacokinetic parameters of TFV were investigated in 2 healthy adult lactating rhesus monkeys which were administered a single 30 mg/kg subcutaneous dose of TFV (m2.6.5, Section 7.3.2.2, P2000116). Following dosing, serum TFV C_{max} values of 18.3 and 30.2 µg /mL were observed in the 2 monkeys. Absorption was rapid, with t_{max} occurring at 0.5 hour. As observed in other species, elimination was biphasic, with apparent half-lives of 3.97 and 2.85 hours for the 2 animals. While this appears shorter than the approximately 9-hour terminal half-life observed in male and nonlactating female monkeys (m2.6.5, Section 3.3.10, P2000031) it may have resulted from the more limited period of sampling (24 vs. 48 hours) in the present experiment.

Serum $AUC_{0-\infty}$ values in this study were 68.9 and 56.2 µg·h/mL for the 2 animals. In comparison to AUC values obtained following 30 mg/kg IV doses of TFV (P2000031), these data suggest essentially complete absorption of TFV after subcutaneous administration.

4.3.3.5. TFV: Immature monkeys

The pharmacokinetic parameters of TFV have been determined in infant rhesus monkeys following subcutaneous administration (m2.6.5, Section 7.3.2.1, 96-DDM-1278-005). Tenofovir was formulated as an aqueous solution and was evaluated in monkeys in 4 age groups (newborn, 1, 3, and 12 months old; n = 2 per group). Tenofovir was administered as a 30-mg/kg injection into the dorsal subcutis region. Plasma samples were obtained over the course of 24 hours and concentrations of TFV were determined by HPLC following fluorescence derivatization. The mean TFV C_{max} values in newborn, 1, 3, and 12-month-old monkeys were 51.8, 30.7, 34.6, and 18.8 µg/mL, respectively; with a t_{max} of 0.5 hour for all age groups. The corresponding plasma clearance (CL/F) of TFV was 0.18, 0.54, 0.41, and 1.02 L/h/kg, respectively, showing an increase in the clearance from birth through 1 year. These results suggest that, at an equivalent dose, younger monkeys received greater TFV exposure. The clearance of TFV was dependent on both the weight and the age of the infant monkeys. It is likely that newborn monkeys lack the anion transport system responsible for tubular secretion of TFV.

4.4. FTC/RPV/TAF: Distribution

Drug interactions, within the FTC/RPV/TAF combination, that affect distribution would not be expected from the data available. While plasma protein binding is high for RPV and moderate for TAF, the binding was very low for FTC and TFV. Therefore, interactions through binding displacement would not be anticipated.

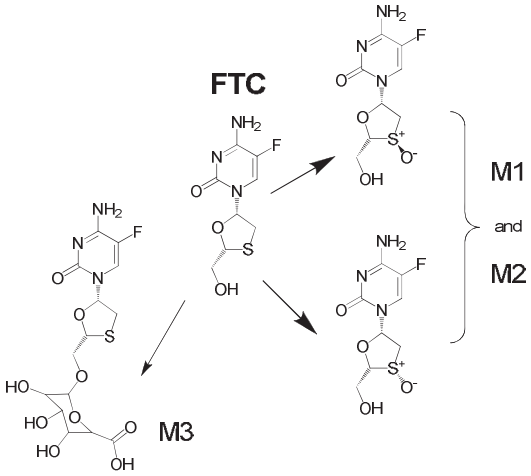
5. METABOLISM

5.1. Proposed Metabolic Pathways

5.1.1. FTC: Metabolic Pathways

Emtricitabine demonstrates high metabolic stability in vitro and in vivo and is the major analyte found in all samples. The metabolites of FTC detected in vitro and in vivo are illustrated in Figure 12.

Figure 12. Pathways for Metabolism of FTC Identified In Vitro and In Vivo



M1 and M2 are diastereomeric sulfoxide metabolites and M3 is a glucuronide conjugate

5.1.2. RPV: Metabolic Pathways

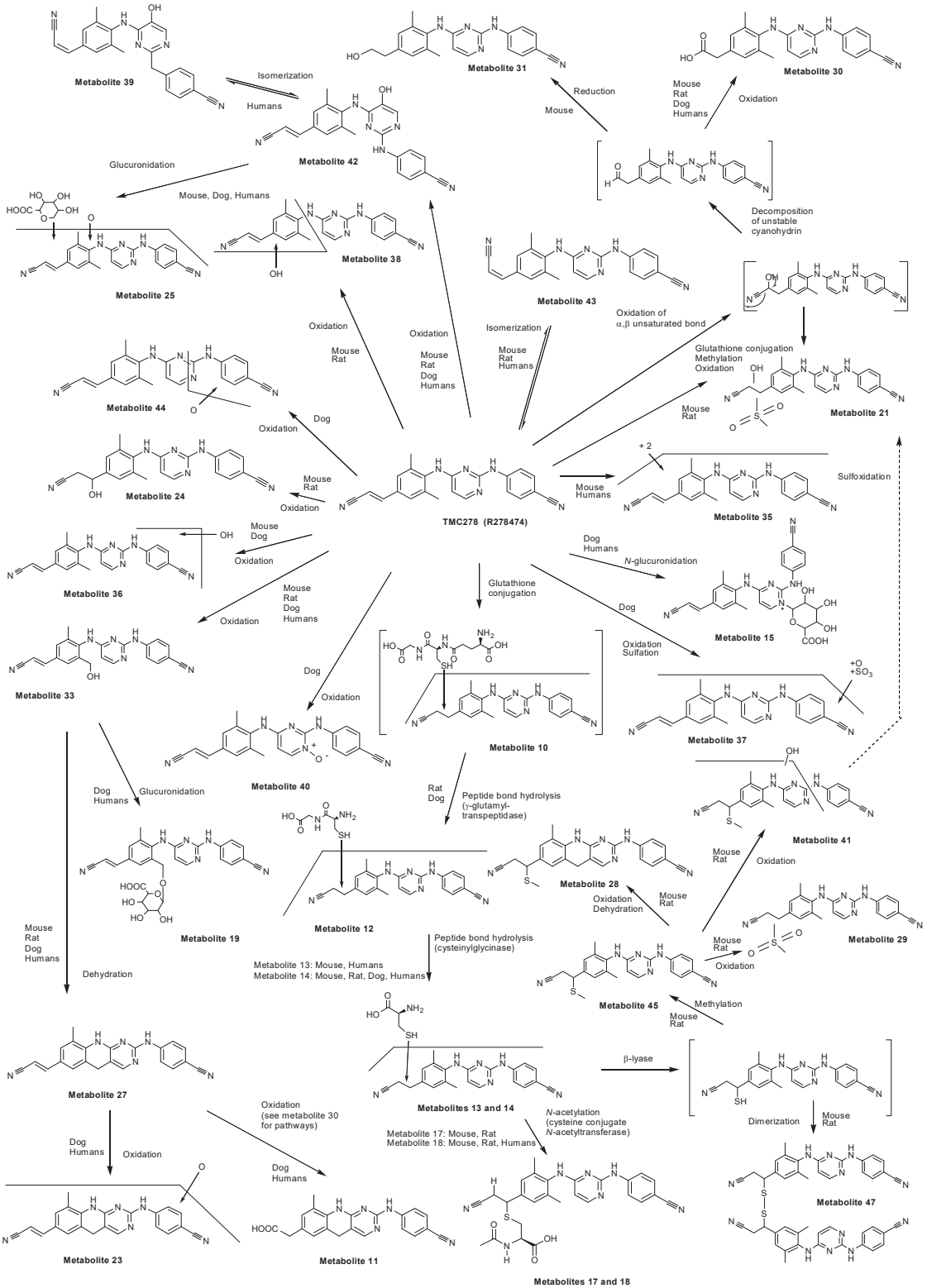
A number of TMC278 metabolites were identified in the in vivo studies in mice, rats, dogs, and humans (m2.6.5, Section 10.2, 2, TMC278-NC190, TMC278-NC113, TMC278-NC114, and TMC278-NC157). The structures of these metabolites and the in vivo metabolic pathways are represented in Figure 13. TMC278 is metabolized via Phase I and Phase II reactions and the most important pathways are hydroxylation and glutathione conjugation. The contribution of the different metabolic pathways to the overall disposition of TMC278 is represented in Table 17.

Table 17. Total Percentage of the Administered Dose Metabolized per Major Pathways in Man and its Corresponding Percentages in Mice, Rats, and Dogs After Oral Administration of [^{14}C]TMC278

	Mice		Rats	Dogs	Man
Metabolites	**20 mg/kg**	**320 mg/kg**	**40 mg/kg**	**5 mg/kg**	**150 mg**
5-Hydroxyl TMC278 at the pyrimidinyl moiety (M42)	18–26 [a]	9.2–13[a]	2.8–3.6[b]	5.3	16
Hydroxymethyl of TMC278 (M33)	0.5–0.7	1.3–1.0	0.54–0.54	8.7 (traces in plasma)	3.0 (seen in plasma)
Carboxylic acid metabolite of the cyanoethenyl moiety (M30)	1.6–3.1	1.5–1.2	0.47 - 0.05	3.1[c]	2.7
Unknown (M35)	< 0.2	< 0.2	—	—	2.2
Tricyclic metabolite (M27) and carboxylic metabolite of M27 (M11)	0.3–< 0.2 [d]	< 0.2–0.1 [d]	0.99–1.60 [f]	3.1[g] (traces of M27 in plasma)	2.2 (M27 seen in plasma)
Glutathione derived conjugates (M13, M14, and M18)	9.6–7.9[e]	8.7–7.3 [e]	0.03–0.46 [i]	< 0.08 [h]	1.2
Unchanged compound	8.8–7.9	33–34	47–43	45	26
N-glucuronide of TMC278 (M15)	—	—	—	traces in plasma	0.6 (seen in plasma)

a co-eluted with M41, M42 was estimated at 13.9-16.6% (20 mg/kg) and at 5.9-8.0% (320 mg/kg)
b co-eluted with M41
c co-eluted with M48
d co-eluted with M28 and M29
e includes M1
f co-eluted with M24, M28 and M29
g including M23
h each of them
i M14 co-eluted with M12
In mice and rats, the first number is male data

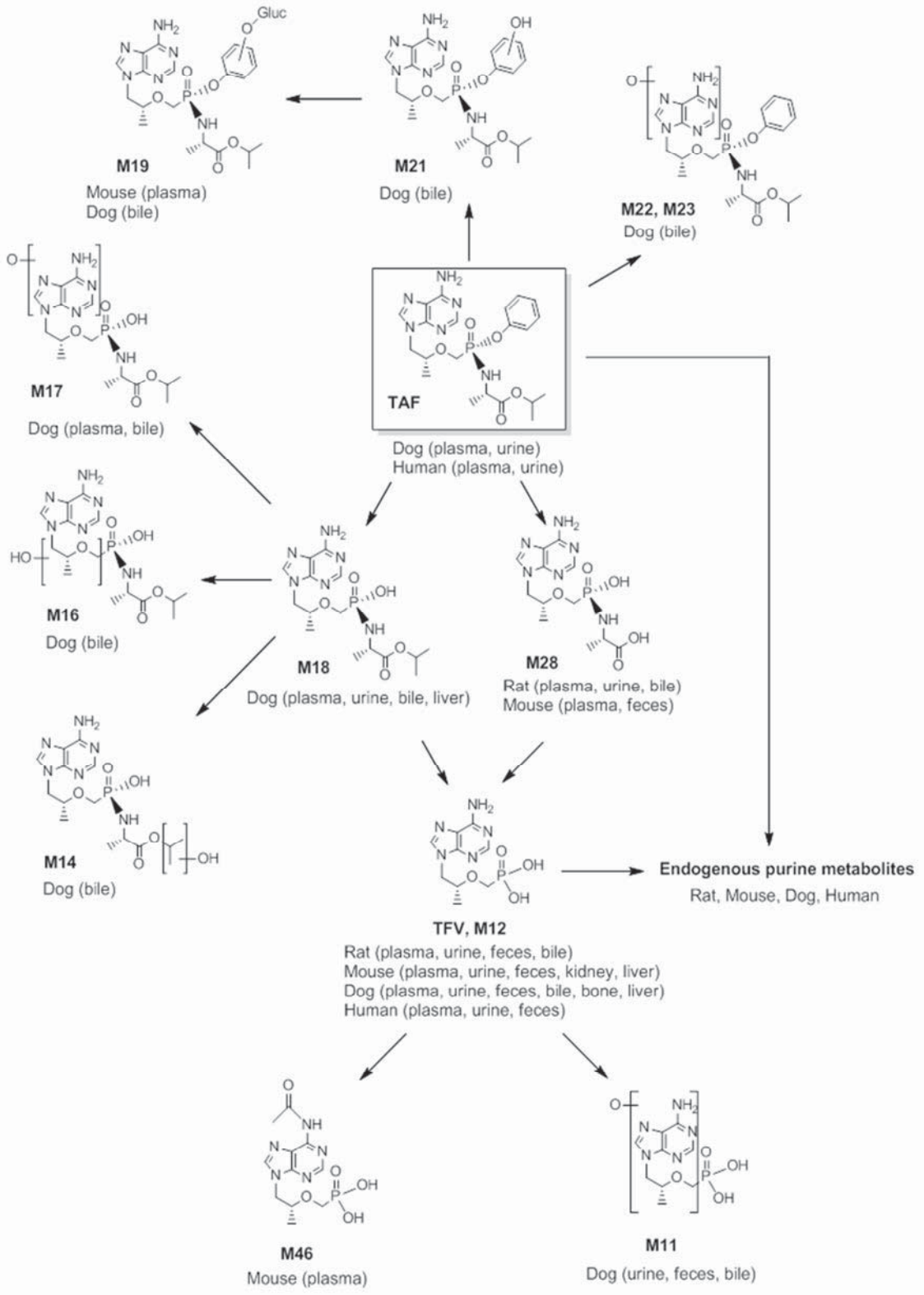
Figure 13. In Vivo Metabolic Pathways of TMC278 in Animals and Humans (Excluding Rat Bile)



5.1.3. TAF: Metabolic Pathways

The metabolic profiles of TAF were determined in plasma, urine, feces, kidney, liver, and nasal turbinate from mice (m2.6.5, Section 10.3.1, AD-120-2012), in plasma, urine, bile, and feces from rats (m2.6.5, Section 10.3.2, AD-120-2021), and in plasma, urine, bile, feces, bone, and liver from dogs (m2.6.5, Section 10.3.3, AD-120-2008). The metabolite profiles were also determined in human plasma, urine, and feces following administration of a single oral dose of [^{14}C]TAF (GS-US-120-0109). Based on the results from mouse, rat, dog, and human, a proposed biotransformation pathway is summarized (Figure 14). Tenofovir alafenamide is also subject to intracellular metabolism to TFV, which is further phosphorylated to the anabolites, tenofovir-monophosphate and TFV-DP (see Table 3) with TFV-DP being the pharmacologically active form.

Figure 14. Metabolites of TAF



5.2. Metabolism In Vitro

5.2.1. FTC: Metabolism In Vitro

An in vitro metabolism study was performed to identify the potential human CYP enzyme(s) responsible for the metabolism of FTC using human liver microsomes and Bactosomes containing cDNA-expressed human CYP enzymes (m2.6.5, Section 8.1.1, 15396v1). The results showed that FTC was relatively stable in the incubation medium. One minor metabolite (~1%) was detected only in incubations with cDNA-expressed CYP3A4 incubations. It was not formed by CYP1A2, 2A6, 2B6, 2D6, 2E1, 2C8, 2C9, or 2C19. Human hepatic microsomal incubations in the presence and absence of selective inhibitors of various CYPs confirmed the low rate of FTC metabolism, and due to incomplete inhibition by the CYP3A-selective inhibitor, ketoconazole, also suggested the possible involvement of FMOs in the metabolism of FTC. In vitro glucuronidation of FTC was not detected.

Pharmacological activation of FTC involves metabolism to FTC-triphosphate, which is a direct binding inhibitor of viral polymerases. The active triphosphate is a very weak inhibitor of mammalian DNA polymerases α, β, ε, and mitochondrial DNA polymerase γ {4241}.

5.2.2. RPV: Metabolism In Vitro

5.2.2.1. In Vitro Metabolic Pathways

The in vitro metabolism of [^{14}C]TMC278 was studied in hepatocytes (suspensions and primary cultures) and liver subcellular fractions (microsomes and 12,000 × g supernatant fractions) of male and female Swiss albino mice; male and female black agouti ras H2 microinjected mice; male and female Sprague Dawley rats; female New Zealand white rabbits; male beagle dogs; and man (m2.6.5, Section 8.2.1, TMC278-NC102). In addition, in vitro metabolism was also studied in hepatocyte primary cultures and 12,000 × g liver supernatant fractions from female Dunking Hartley guinea pigs and female or male cynomolgus monkeys (m2.6.5, Section 8.2.2, TMC278-NC333). TMC278 (5 μM) was incubated in the above systems at 37°C for various time periods. Incubates were analyzed for metabolites by radio-HPLC. Co-chromatography, enzyme hydrolysis, LC-MS/MS, and nuclear magnetic resonance (NMR) techniques were used for the identification of metabolites.

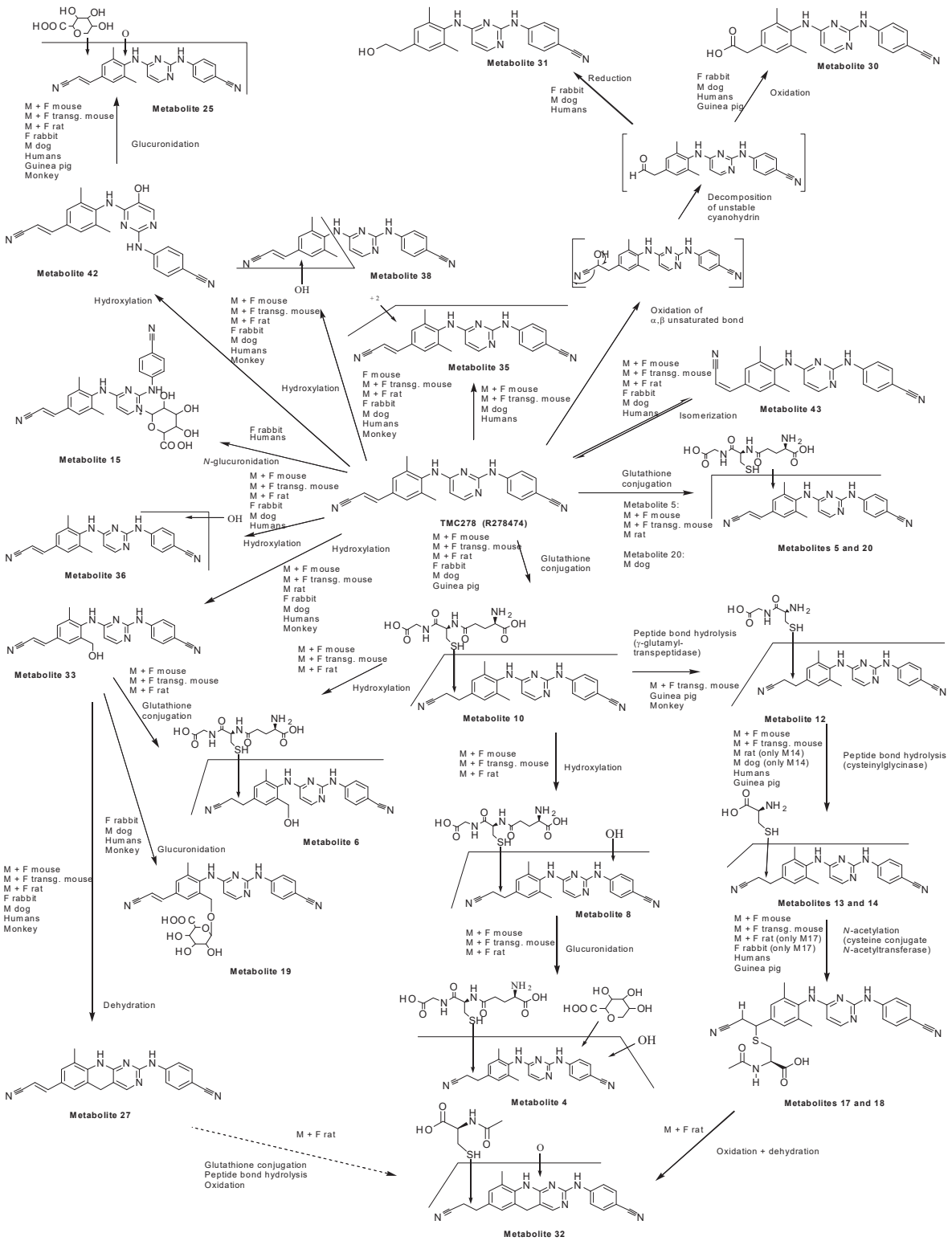
In each species, a large number of metabolites was detected (see Figure 15). Overall, TMC278 was metabolized via different metabolic pathways including aromatic and aliphatic hydroxylation; glutathione conjugation; N-glucuronidation; nitrile release followed by reduction/oxidation; and isomerization. Aromatic hydroxylation at the pyrimidinyl moiety (M42) subsequently followed by glucuronidation (M25) was an important metabolic pathway in all the species, and it was the most important in vitro biotransformation route in human, dog, and rabbit. Aliphatic hydroxylation at one of the methyl groups of the cyanoethenyl-2,6-dimethylphenyl moiety (M33), subsequently followed by dehydration to form a tricyclic metabolite (M27), proved to be an important metabolic pathway in human, monkey, and rabbit, but was less important in the other animal species. The combination of aliphatic hydroxylation with glutathione conjugation (M6) occurred in the mouse strains and in male and female rats, but not

in the other species. Aliphatic hydroxylation in combination with glucuronidation (M19), on the contrary, was observed in rabbit, dog, monkey, and human, but not in mouse, rat, and guinea pig. Glutathione conjugation, subsequently followed by conversions leading to mercapturic acid metabolites (M17 and M18), was a main metabolic route in mouse, rat, and guinea pig. In the other species, the mercapturic acid biosynthesis route proved to be a minor pathway, and not all intermediary metabolites were detected. Hydroxylation of the glutathione conjugate (M8), subsequently followed by glucuronidation, was also solely observed in mouse and rat. The release of the nitrile group followed by reduction/oxidation, resulting in the formation of an alcohol metabolite (M31) and a carboxylic acid metabolite (M30), was a minor metabolic pathway in rabbit, guinea pig, dog, and human, and could not be detected in mouse, rat, and monkey. N-glucuronidation at the pyrimidinyl moiety of TMC278 (M15) was an important biotransformation pathway in rabbit and could also be detected in human, but not in the other species.

All identified TMC278 metabolites that were detected in human in vitro systems were also detected in at least 1 animal species.

In a previous in vitro metabolism study with cold TMC278 (TMC278-FK4152), glutathione conjugation of TMC278 was identified as the most important metabolic pathway in man and rodents. This was not confirmed in the in vitro study with [^{14}C]TMC278 and in vivo where hydroxylation was the most important metabolic pathway in man.

Figure 15. In Vitro Metabolic Pathways of TMC278 in the Liver of Swiss Albino Mouse, Sprague Dawley Rat, Guinea Pig, Rabbit, Dog, Monkey, Man and Black Agouti Ras H2 Mouse



5.2.2.2. Isozymes Involved in the Metabolism of TMC278 in Human Liver

CYP450 Isozymes Involved in TMC278 Metabolism:

The in vitro metabolism of [^{14}C]TMC278 was studied in human liver microsomes (HLM) in the presence of a nicotinamide adenine dinucleotide phosphate (NADPH)-generating system (m2.6.5, Section 8.2.4, TMC278-NC141). The CYP reaction phenotyping of TMC278 metabolism was performed by different approaches, including effect of CYP diagnostic inhibitors on TMC278 metabolism, metabolism in expressed CYP systems (*E coli* cells and Supersomes®), and correlation analysis of metabolism rate in a panel of 10 batches of characterized HLMs. Incubations were conducted at various TMC278 concentrations (0.5–50 µM) for 15 minutes with a protein concentration of 0.25 mg/mL. In a preceding nonradiolabeled pilot metabolism study, the identification of CYP isoenzymes was based on inhibitor and metabolism experiments with heterologous expression systems (TMC278-FK4151).

In the radiolabeled study, 1 primary TMC278 metabolite, M42, and 4 minor metabolites (i.e., M33, M27, and the co-eluting metabolites M35 and M36) were formed. The apparent Michaelis-Menten constant K_m and V_{max} values for the metabolism of TMC278 in HLMs were 4.17 µM and 381 pmol/mg/min, respectively. The use of different CYP diagnostic inhibitors showed that TMC278 metabolism was markedly inhibited by the different CYP3A diagnostic inhibitors. Formation of M33 was moderately inhibited with the CYP2C8/9/10 inhibitor sulphaphenazole. Metabolism experiments in expressed CYP P450 *E coli* and Supersomes® systems clearly indicated the involvement of CYP3A isoforms and to some extent of the CYP1A2 isoform. Correlation analysis showed involvement of CYP3A and CYP2C19 in the formation of several metabolites, though for CYP2C19 this was not confirmed in the other phenotyping experiments. CYP1A2 might also play a role in the formation of M33. During some of the experiments in this study, the recovery of the total radioactivity was around 70% in the presence of NADPH and cofactor. Addition of glutathione to the incubation mixture resulted in a 50% decrease of bound radioactivity. This suggests that glutathione was able to scavenge hypothetical reactive intermediates. Comparison of the metabolic profile of TMC278 after incubation in HLMs in the absence and presence of glutathione confirmed the formation of several glutathione conjugates.

In conclusion, overall TMC278 metabolism, as well as formation of all its metabolites, were mainly catalyzed by CYP3A4. Additionally, it was observed that formation of certain metabolites could also be catalyzed to a lesser extent by CYP2C19, CYP1A2, and CYP2C8/9/10.

In the nonradiolabeled metabolism study, CYP3A4 was clearly involved in the metabolism of TMC278 based on both inhibition and metabolism data in heterologous expression systems. Metabolism experiments with heterologous expression systems also indicated the possible involvement of CYP1A1, CYP1B1, CYP2C18, and CYP3A5 in the metabolism of TMC278.

In an earlier study, using non-radiolabeled compound, a K_m value of 4.94 µM and a V_{max} value of 0.84 nmol/mg/min was calculated. Based on these kinetic parameters, a human in vivo intrinsic clearance of 1410 L/h and a hepatic clearance of 0.042 L/h/kg were predicted (TMC278-FK4288).

GST Isoforms Involved in TMC278 Metabolism:

The identification of the glutathione S-transferase (GST) isoforms (alpha, mu, and pi) involved in the metabolism of [^{14}C]TMC278 was studied in vitro using heterologous expressed GST (TMC278-FK4789). [^{14}C]TMC278 was tested at 5 and 200 µM using reduced glutathione (GSH, 1 mM) as co-substrate. In addition, incubations were performed in the absence of GST to estimate the amount of nonenzymatic conjugation.

Conjugation with glutathione was more dependent on the mu than the pi isoform of GST, although both isoforms were involved.

5.2.2.3. Enzyme Induction and Inhibition

In Vitro Study Measuring CYP Activity and CYP mRNA Induction in Human Hepatocytes:

The potential of TMC278 to induce CYP450 activities was determined in primary cultures from cryopreserved human hepatocytes originating from 3 different donors and compared to the data obtained with the positive controls omeprazole, rifampicin, and ethanol (m2.6.5, Section 13.2.4, TMC278-NC186). Cells were treated for 2 consecutive days either with vehicle (DMSO), with TMC278 (2.5, 10, and 25 µM), or with the CYP inducers, i.e., omeprazole (CYP1A2), rifampicin (CYP2B6/2C19/3A4), or ethanol (CYP2E1). Induction of CYP activities (CYP1A2, CYP2B6, CYP2C19, CYP2E1, and CYP3A4) was assessed at the end of the 48-hour treatment period using corresponding probe substrates (phenacetin (CYP1A2), S-mephenytoin (CYP2B6 and CYP2C19), chlorzoxazone (CYP2E1), and testosterone (CYP3A4). LC-MS/MS was used to measure the products of the probe substrates in order to determine the CYP activity of the hepatocytes. In addition, induction of CYP activities was also determined by measurement of mRNA expression levels by TaqMan real-time reverse transcription-polymerase chain reaction.

Most of the batches responded well to the treatment of positive inducers in all assays, except for the CYP2E1 assay. However, the inhibition control (positive control + 25 µM TMC278) revealed that TMC278 seemed to mask the induction of all investigated CYPs. Based on the observed fold-changes of mRNA expression and fold-induction of CYP activities, it can be concluded that TMC278 might be a very weak inducer of CYP1A2 (6-fold less than omeprazole) and CYP2B6 (4.5-fold less than rifampicin) in human hepatocytes. In addition, the results indicate that TMC278 appears to be a moderate inducer of CYP2C19 (1.4-fold less than rifampicin) and CYP3A4 (2-fold less than rifampicin) in human hepatocytes. No conclusion could be drawn for CYP2E1.

In Vitro Study Measuring GST Activity Induction in Human Hepatocytes:

The potential of TMC278 to induce GST was evaluated in 1 batch of primary human hepatocytes in the presence of 3 concentrations of TMC278 (1, 10, and 30 µM), incubated for 3 consecutive days (TMC278-FK4824).

TMC278 had a low or no effect on GST activity or GST-alpha and GST-mu immunoreactive protein levels when compared with enzyme rates or levels observed in hepatocytes treated with the vehicle control DMSO. However, the positive controls (phenobarbital, rifampin, or

2,3,7,8-tetrachlorodibenzo-p-dioxin) did not result in induction of GST in human hepatocytes and therefore no conclusions can be drawn on the inducing properties of TMC278 on the GST activity and expression from this study.

Ex-vivo Studies Measuring Enzyme Activities in Mouse, Rat, and Dog Liver:

TMC278 in aqueous HPMC (0.5%) was administered for 3 months to male and female CD-1 mice at doses of 20, 80, and 320 mg/kg/day (m2.6.5, Section 13.2.1, TMC278-NC192). TMC278 base in PEG400/CA (10%) was administered to male and female Sprague Dawley rats at doses of 40, 120, and 400 mg/kg/day and to male and female beagle dogs at doses of 5, 10, and 40 mg/kg/day for 6 months (m2.6.5, Sections 13.2.2 and 13.2.3, TMC278-NC193 and TMC278-NC140, respectively).

To examine the effect of TMC278 on some hepatic enzyme activities, microsomal fractions of livers from the above mentioned TMC278-treated animals were assayed for protein and total CYP content, and for the activities of 7-ethoxyresorufin O-deethylase, 7-pentoxyresorufin O-dependentylase, 4-nitrophenol hydroxylase, testosterone 6β-hydroxylase, and lauric acid 12-hydroxylase. These enzyme activities are well known markers for the induction of CYP1A, CYP2B, CYP2E, CYP3A, and CYP4A forms, respectively. Microsomes were also assayed for lauric acid 11-hydroxylase activity, which is largely catalyzed by CYP2E1 and for thyroxine UDP-GT activity. Additionally, liver cytosolic fractions were assayed for protein content and GST activity towards 1-chloro-2, 4 dinitrobenzene as a substrate. In addition, in rats, the effect of TMC278 on some hepatic enzyme activities were also examined in liver samples from a 2-week study (TMC278 base in PEG400/CA (10%) at 40, 120, and 400 mg/kg/day) (TMC278-FK4247). In this study, the liver microsomes were assayed for protein and for 7-ethoxyresorufin *O*-deethylase (CYP1A1, CYP1A2), 7-pentoxyresorufin-dealkylase (CYP2B), aniline hydroxylase (CYP2E1), *N*-ethyl morphine *N*-demethylase (CYP3A1, CYP3A2), lauric acid hydroxylase (CYP4A1), and thyroxine (T4) glucuronosyltransferase activities.

In mice, TMC278 was an inducer of the CYP4A forms in both male and female animals (up to 25- and 20-fold, respectively) (see Table 18). Some induction was also seen with the CYP3A forms (up to 1.7-fold in both males and females). TMC278 treatment induced UDP-GT activity in male and female mice (up to 2.1- and 2.3-fold, respectively) and decreased GST activity in male mice to 44% at 320 mg/kg/day.

In rats, TMC278 was an inducer of CYP4A forms in male rats (4.7-fold), whereas in female rats, TMC278 was an inducer of CYP3A forms (6-fold) and possibly also of CYP2B and CYP4A forms (see Table 18). TMC278 treatment had some effect on UDP-GT activity in male rats (induction of 1.3 fold only at high dose level) and on GST activity in female rats (induction of 1.5-fold). In the 2-week study, the results were similar.

In dogs, treatment with TMC278 did not result in any induction of CYP1A1, CYP2B, CYP2E, CYP4A, UDP-GT, or GST activity. TMC278 produced some decrease in microsomal CYP3A-dependent testosterone 6β-hydroxylase activity, but this effect was confined to the 2 highest dose levels and was not dose-dependent (see Table 18).

Table 18. Percentage of Testosterone 6β-hydroxylase, Lauric Acid 12-hydroxylase and UDP-GT Activities Relative to Control Values in Hepatic Microsomal Fractions of CD-1 Mouse, Sprague Dawley Rat and Beagle Dog After Repeated Administration of TMC278 or TMC278 Base

Species	Dose (mg/kg/day)	Testosterone 6β-hydroxylase (CYP3A)		Lauric Acid 12-hydroxylase (CYP4A)		Thyroxine UDP Glucuronosyltransferase (UDP-GT)	
		Male	Female	Male	Female	Male	Female
Mouse (TMC278-NC192)	20	111	153**	147*	126	108	138*
	80	156***	174***	525***	521***	150**	164***
	320	174***	175***	2499***	1966***	210***	229***
Rat (TMC278-NC193)	40	95	120	140	75	65**	127
	120	125	300***	262**	93	77*	98
	400	120	600***	466***	127*	125*	134
Dog (TMC278-NC140)	5	85		100		82	
	10	57**		102		75	
	40	74*		113		68	

* p<0.05; ** p<0.01; *** p<0.001

In Vitro Inhibition of Human CYP450 Enzymes by TMC278:

TMC278 was tested for its inhibitory effect on the metabolism of various human CYP450 probe substrates to gain information about the possibility of clinically relevant interactions with other drugs (TMC278-FK4123 and TMC278-NC283). Incubations with P450 probe substrates, selective towards CYP1A2, CYP2A6, CYP2C8/9/10, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP4A, and CYP3A4/5 were performed in HLM in the absence and presence of TMC278 at 8 different concentrations ranging between 0.03 and 400 µM (Table 19).

Table 19. Interaction of TMC278 With Human CYP450 In Vitro

Substrate	CYP Involved	IC_{50}-value (µM)
Phenacetin	CYP1A2	34.0
Coumarin	CYP2A6	> 100 (15.7)[a]
Tolbutamide	CYP2C8/9/10	3.99
Dextromethorphan	CYP2D6	3.88
Bufuralol	CYP2D6	12.0
Testosterone	CYP3A4	6.29
Cyclosporin A	CYP3A4	16.8
Midazolam	CYP3A4/5 CYP3A4/5	4.20 18.3
Lauric acid	CYP4A CYP2E1	> 100 (15.9) [a] 9.79

a % inhibition at 100 µM

Under the conditions of this experiment, TMC278 was a potent inhibitor of CYP2C19 and CYP2E1. CYP2C19 activity was blocked 70% at a concentration of 0.06 µM (0.02 µg/mL), and 86% of CYP2E1 activity was inhibited at a concentration of 0.03 µM (0.01 µg/mL). However, an in vitro study with cultured hepatocytes (see Section 5.2.2.3) indicated a moderate induction of CYP2C19 by TMC278.

For CYP2E1, there were some discrepancies in this in vitro study. TMC278 seemed to be a strong inhibitor of CYP2E1 with chlorzoxazone as a substrate and not with lauric acid as a substrate. However, in the in vitro drug-drug interaction study (see Section 7.2) and also in the in vivo clinical study (m2.7.2, Section 3.3.3.2.1, [Clinical Study C139]), no interaction was observed between TMC278 and chlorzoxazone. Therefore, as it was not confirmed by subsequent studies, the inhibition of CYP2C19 and CYP2E1 by TMC278 is considered not relevant. For the other CYPs, taking into account a mean C_{max}-value of about 0.13 µg/mL for TMC278 in human, inhibition in vivo is unlikely.

Inhibition of CYP2C8-mediated paclitaxel 6α-hydroxylation and CYP2C9-mediated S-warfarin-7-hydroxylation by TMC278 (0.1 - 300 or 200 µM, respectively) was also investigated in HLMs (TMC278-NC283). TMC278 is an inhibitor of CYP2C8 and CYP2C9 with a K_i of 10 and 1.7 µM, respectively. Taking into account a mean C_{max}-value of about 0.13 µg/mL for TMC278 in human, inhibition of CYP2C8 and CYP2C9 by TMC278 is not expected ($C_{max}/K_i<0.1$).

Effect of TMC278 on Adrenal Gland:

The effect of TMC278 on cortisol biosynthesis in dog adrenal cortex cell-free extracts was determined (TMC278-FK4790) (see also m2.6.6, Section 8.3.2.1.2).

TMC278 at a nominal concentration of 75 µM (27.75 µg/mL) caused 39% inhibition of the metabolism of pregnenolone compared to control. A concentration-dependent increase in progesterone and 17α-hydroxyprogesterone concentrations was noted concomitant with decreases of 11-deoxycorticosterone, 11-deoxycortisol, and corticosterone concentrations.

5.2.3. TAF and TFV: Metabolism In Vitro

Stability of TAF was assessed in plasma, intestinal S9, and hepatic S9 fractions from dogs and humans (m2.6.5, Section 8.3, AD-120-2023, AD-120-2024, AD-120-2025, and m2.6.5, Section 14.3.5, AD-120-2027). Tenofovir alafenamide was moderately stable in plasma and intestinal S9 with half-lives of 74.7 and 58.3 minutes for human and 69.5 and 47.1 minutes for dog, respectively (m2.6.5, Section 8.3.1, AD-120-2025 and m2.6.5, Section 8.3.3, AD-120-2024). The stability of TAF in human intestinal S9 fractions was also determined in a separate study assessing the effect of HIV-PIs on TAF stability in intestinal S9 (discussed in Section 7.3.2) and a somewhat lower but similar half-life for TAF was observed (24.5 minutes; AD-120-2027). Relative to plasma or intestinal S9, TAF was somewhat less stable in human and dog hepatic S9 fractions with half-lives of 20.6 and 31.1 minutes, respectively (Table 20). Based on these data, predicted hepatic extraction ratios for human and dog were calculated to be 76.2% and 60.5%, respectively (m2.6.5, Section 8.3.2, AD-120-2023).

Table 20. Stability of TAF in Biological Matricies from Dog and Human

Species	TAF Stability, $t_{½}$ (min)		
	Plasma	Intestinal S9	Hepatic S9
Human	74.7	24.5-58.3	20.6
Beagle Dog	69.5	47.1	31.1

The potential for CYP enzymes to metabolize TAF was assessed by incubating TAF with 6 individual bacterially expressed human CYP enzyme preparations (Bactosomes) coexpressed with human NADPH CYP reductase (m2.6.5, Section 8.3.4, AD-120-2004). Metabolism of TAF was not detected by CYP1A2, CYP2C8, CYP2C9, CYP2C19 or CYP 2D6. Tenofovir alafenamide was slowly metabolized by CYP3A4 at a rate of 1.9 min^{-1} which was 26.6% of the positive control, testosterone.

Intracellular metabolic activation of TAF in PBMCs or other lymphatic tissues involves conversion to TFV by cathepsin A (m2.6.2. Section 2.2.1) {10427}, {13119}. In contrast to PBMCs, TAF was primarily hydrolyzed by carboxylesterase 1 (CES1) in primary hepatocytes as described in Section 7.3.2. Tenofovir is then further phosphorylated to TFV-DP by cellular nucleotide kinases. These steps are high capacity and low affinity and are not readily inhibited by other xenobiotics.

The in vitro activation of TAF in human primary hepatocytes was evaluated and compared with that of TDF and TFV (m2.6.5, Section 8.3.5, AD-120-2017). Following a 24-hour continuous incubation of primary hepatocytes with 5 µM TAF, TDF, or TFV, the levels of GS-77938 increased to 1,470, 302, and 12.1 pmol/million cells illustrating that incubation with TAF resulted in 5- and 120-fold higher intracellular levels of GS-77938 compared to TDF and TFV, respectively.

The in vitro metabolism of [^{14}C]TFV was studied in dog plasma, in control and induced (Aroclor™ 1254) rat liver microsomes, and also in dog liver and intestinal S9 fractions (m2.6.5, Section 8.3.6, 96-DDM-1278-003). Potential isomerization of TFV was determined using a chiral HPLC assay with radioactive flow detection. Radioactivity associated with the protein pellet was also determined by sample oxidation and liquid scintillation (< 0.1% of radioactivity). Tenofovir was recovered unchanged under all conditions: no metabolites were detected in either rat microsomal preparation, with or without the addition of NADPH cofactor. There was no evidence of chiral inversion. Similarly, there was no apparent loss of TFV following incubation with dog plasma, liver, or intestinal S9 fractions, and no metabolites were detected.

5.3. Metabolism In Vivo

5.3.1. FTC: Metabolism In Vivo

5.3.1.1. FTC: Mice

The metabolism and elimination of orally administered [^{3}H]FTC was studied in male CD-1 mice (m2.6.5, Section 10.1.1, TEIN/93/0015). Urine and feces were assayed by LSC and HPLC. Urinary recovery of radioactivity was 67% ± 7% of the dose. In the feces, 18% ± 3% of the dose was recovered, all as unchanged FTC. Total recovery of radioactivity excreted in urine and feces was 85% ± 4% of dose. In the urine, 64% ± 7% of the radioactivity was recovered as unchanged FTC in the 0- to 24-hour sample. Three metabolites of FTC were measurable in the urine. These metabolites were tentatively identified as: M1 and M2 (2 isomeric, 3'-sulfoxides of FTC, 1.7% ± 0.3% and 2.0% ± 0.4% of dose recovered, respectively); and 5-fluorocytosine (1.4% ± 0.1% of dose recovered). Traces of 5 other metabolites and a peak tentatively identified as tritiated water were also observed at levels of less than 1% of dose. 不純物FA* was not observed (< 0.1% of dose).

5.3.1.2. FTC: Rats

The urine collected from male Sprague-Dawley rats following IV or oral administration of 10 mg/kg [^{3}H]FTC was subject to radiochromatographic profiling {4570}. Metabolite profiles were independent of the route of administration and revealed that FTC accounted for the majority of the radioactivity, with the sulfoxides (M1 and M2) being the most abundant metabolites. Small amounts were accounted for by the glucuronide (M3), 5-fluorocytosine, and tritiated water. 不純物FA* was not detected (< 0.1% of the dose).

5.3.1.3. FTC: Monkeys

An exploratory study was performed with [^{3}H]FTC administered orally to female cynomolgus monkeys (m2.6.5, Section 10.1.3, TEIN/93/0016). By 72 hours postdose, 41% of radioactivity was recovered in urine, 33% in feces, and 10% in cage washings. Radiochromatographic analysis revealed that parent FTC accounted for the majority of the radioactivity in all samples (64% of urinary radioactivity and 98% of fecal radioactivity). A sulfoxide metabolite (M1 or M2) represented the majority of nonFTC radioactivity in urine and totaled 11% of the dose.

A further in vivo metabolism study was performed in cynomolgus monkeys after oral administration of [^{14}C]FTC (m2.6.5, Section 10.1.2, TOX063). Results were very similar to the previous study. Parent FTC accounted for the majority of the radioactivity in urine (74%) and feces (97%). The majority of the nonFTC radiolabel in urine was accounted for by metabolites M1, M2, and M3. Another potential urinary metabolite, 不純物FA* , was not detectable (< 0.02% of dose).

5.3.2. RPV: Metabolism In Vivo

5.3.2.1. RPV: Mice

Male and female CD-1 mice were dosed orally, by gavage, with a single dose of [^{14}C]TMC278 base in PEG400/CA (10%) at 20 or 320 mg/kg (m2.6.5, Section 10.2.1, TMC278-NC190). Plasma samples were collected up to 24 hours after dosing and urine and feces samples up to 96 hours after dosing. Radioactivity was determined by scintillation counting and samples were analyzed by radio-HPLC. Plasma concentrations of TMC278 were measured by LC-MS/MS and the metabolite profiling and identification were done by radio-HPLC and LC-MS/MS analyses.

After oral administration of [^{14}C]TMC278 base, 87% to 96% of the total radioactivity was eliminated in feces and 1.8% to 4.2% was excreted in urine. Unchanged TMC278 accounted for 7.9% to 8.8% and 33% to 34% of the administered dose in the overall pooled 0- to 48-hour feces at 20 and 320 mg/kg, respectively. Renal elimination was limited, only traces of unchanged TMC278 were excreted in urine (0.02% to 0.63% of the dose) at the 2 dose levels.

TMC278 was extensively metabolized in the mouse, as a large number of metabolites were detected (Figure 13). Metabolic pathways included oxidative pathways (aliphatic and aromatic hydroxylation) and glutathione conjugation, followed by secondary metabolism (metabolism of the glutathione conjugate, glucuronidation, dehydration).

In feces, by far the predominant metabolite fraction was composed of M41 (hydroxy metabolite of S-methyl conjugate of TMC278) and M42 (aromatic hydroxylation at the 5-position of the pyrimidinyl moiety), accounting for 18% to 26% of the 20 mg/kg dose and for 9% to 13% of the 320 mg/kg dose. In this fraction, M42 was the most abundant metabolite for both genders and at the 2 dose levels, estimated at about 14% and 17% of the 20 mg/kg dose, and at 5.9% and 8.0% of the 320 mg/kg dose in male and female mice, respectively. The cysteinyl conjugates (M13 co-eluted with M14) and the mercapturic acids (M17 co-eluted with M18) accounted for 6.2% to 9.2% of the dose in total (2 dose levels, both genders). M24 (hydroxylation on the cyanoethenyl moiety) co-eluted with M25 (oxidation followed by glucuronidation of TMC278) and accounted for less than 3.4% in total (2 dose levels, both genders). M30 (carboxylic acid metabolite on the cyanoethenyl moiety) accounted for about 1.6% to 3.1% of the 20-mg/kg dose and about 1.2% to 1.5% of the 320-mg/kg dose. Several minor metabolites in both male and female mice were present: M38 (hydroxylation of TMC278) and M43 (Z isomer of TMC278) that co-eluted with M45 (S methyl conjugate) accounted for less than 2.2%. M21 (hydroxylated methyl sulphonyl conjugate) and M33 (hydroxylation at the methyl group of TMC278) accounted for less than 1.4%. M27 (tricyclic metabolite) co-eluted with M28 (tricyclic metabolite of S-methyl conjugate of TMC278) and M29 (sulphoxidation of M45); and M47 (dimer) accounted for less than 0.8%. M35 and M46 (unknown structures) accounted for less than 0.7%.

In urine, M25 (oxidation in combination with glucuronidation) was the most abundant metabolite at both dose levels and accounted for 0.4% to 1.6% of the dose. M13, M14, M17, and M18 were more formed in female mice than in male mice and accounted for 1.2% and 1.1% of the dose in females (at 20 and 320 mg/kg, respectively) and for 0.54% and 0.27% of the dose in males (at 20 and 320 mg/kg, respectively). M42 was also excreted in urine (0.06% to 0.37% of the dose).

In plasma, unchanged TMC278 was by far the main circulating compound at both dose levels. At all time points, M33 (hydroxymethyl TMC278) was the most abundant plasma metabolite; it accounted for 1.5% to 6.6% of the plasma radioactivity. Other metabolites (M13 co-eluted with M14, M27, M30, and M36) accounted for less than 1.3% of the sample radioactivity. Traces of several other metabolites (M17, M18, M31 [hydroxyl metabolite on the cyanoethenyl moiety], M42, and M43) were detected.

The metabolite profile of TMC278 was qualitatively comparable in male and female mice.

5.3.2.2. RPV: Rats

Single Dose Administration:

Male and female Sprague Dawley rats were dosed orally, by gavage, with a single dose of [^{14}C]TMC278 base in PEG400/CA (10%) at 40 mg/kg (m2.6.5, Section 10.2.2, TMC278-NC113). Plasma samples were collected up to 24 hours after dosing, and urine and feces samples were collected up to 96 hours after dosing. Plasma concentrations of TMC278 were measured by LC-MS/MS. The biliary metabolite profile of TMC278 was also investigated in male Sprague Dawley rats after a single dose of [^{14}C]TMC278 base in PEG400/CA (10%) at 40 mg/kg (m2.6.5, Section 10.2.3, TMC278-NC145). In both studies, the total radioactivity was measured by scintillation counting. Metabolite profiles were determined by reversed-phase radio-HPLC, and identification of the metabolites was carried out using co-chromatography with synthesized metabolites, enzymatic hydrolysis, and LC-MS/MS analysis.

After oral administration of [^{14}C]TMC278 base, the radioactivity was predominantly excreted in feces (93% of the dose), and 0.45% (males) to 1.8% (females) was excreted in urine. Unchanged TMC278 accounted for 47% and 43% of the administered dose in the overall pooled 0- to 48-hour feces in males and females, respectively. Urinary excretion of unchanged TMC278 was negligible.

TMC278 was metabolized to a moderate extent. Metabolic pathways included oxidative pathways (aliphatic and aromatic hydroxylation), glutathione conjugation, and metabolites derived thereof (Figure 13).

In feces, dimerization of a thiol intermediate resulting from glutathione conjugation (M47) was the most predominant metabolic pathway in male and female rats (4.0% and 3.8% of the dose, respectively). M41/M42 and M43/M45, which co-eluted pairwise, resulted from hydroxylation, isomerization, or glutathione conjugation and accounted on average for 2.4% to 3.6% of the dose in both genders. A variety of minor metabolites (M21, M24/M27/M28/M29, M33, M38, and M46) were also observed in rat feces; each represented less than 2% of the administered dose. M30 accounted for 0.47% and 0.05% of the dose in male and female rats, respectively.

In urine, only the mercapturic acids (M17 and M18) were detected and accounted for 1.1% and 0.45% in female rats and for 0.02% and 0.03% of the dose in male rats, respectively. Several unknown metabolites were also present in urine but accounted in total for < 0.4% of the dose.

In plasma, unchanged TMC278 accounted for the largest fraction of the circulating radioactivity. Only 2 minor metabolites, M12, a cysteinylglycine-S-conjugate, which co-eluted with M14, a cysteinyl-S-conjugate, were present in plasma. They accounted for 4% to 14% of the plasma radioactivity in total, at all time points.

The metabolic profile of TMC278 is qualitatively comparable in male and female rats.

In bile, the amount of radioactivity excreted within 24 hours after a dose of [^{14}C]TMC278 base was rather low, i.e., 18% and 25% of the radioactive dose, in restrained and nonrestrained rats, respectively. The percentage of unchanged TMC278 excreted in the bile during this time period was negligible (~0.2%). The most important biotransformation pathway involved conjugation of glutathione to TMC278 to form M10 followed by formation of the cysteinylglycine-S-conjugate M12 and the cysteine-S-conjugate M14. They accounted for 6.4% in total. From this pathway, other metabolites were generated. The most abundant ones were M9 (thiol glucuronide conjugate) and M18 (mercapturic acid S-conjugate of TMC278) and each accounted for less than 2.8%. The minor one was M1 (oxidation and glucuronidation of M14) and accounted for 0.63%. Other minor metabolites were M25 (1.4%) and M30 (0.4%) (m2.6.5, Section 10.2.3, TMC278-NC145).

Multiple Dose Administration:

In an attempt to understand the time-dependent decrease in exposure in male rats (see Table 11), the metabolic profiles of plasma samples obtained after oral administration of TMC278 at 1500 mg/kg/day in male and female rats (carcinogenicity study) were assessed by LC/UV and LC/MS (TMC278-NC290; m2.6.5, Section 4.2.12, TMC278-NC123; see Section 3.3.2.2).

In all samples, unchanged TMC278 was by far the major circulating compound. The comparison of the plasma profiles from male and female rats at Day 1, Week 27, and Week 39 did not show a relevant increase in metabolites after repeated administration.

5.3.2.3. RPV: Dogs

Male beagle dogs were dosed orally with a single dose of [^{14}C]TMC278 base in PEG400/CA (10%) at 5 mg/kg (m2.6.5, Section 10.2.4, TMC278-NC114). Plasma, urine, and feces were collected up to 168 hours (1 week) after dosing. Plasma concentrations of TMC278 were measured by LC-MS/MS. Radioactivity levels were determined by liquid scintillation counting and metabolite profiles were investigated by radio-HPLC and LC-MS/MS.

After oral administration of [^{14}C]TMC278 base, radioactivity was excreted predominantly in feces (95% of the dose), while a very low amount of radioactivity was excreted in urine (1.7% of the dose). Unchanged drug represented 45% of the administered dose in the overall pooled 0- to 72-hour feces. Unchanged TMC278 was not detected in urine.

TMC278 was not extensively metabolized in dogs. The most important biotransformation pathway of TMC278 in dogs was oxidation at various positions of the molecule. In addition, but less significant, direct N-glucuronidation of TMC278 and further metabolism of the oxidized metabolites via dehydration (ring closure), glucuronidation, and sulfation occurred (Figure 13).

The most abundant fecal metabolites included M33 (hydroxymethyl-TMC278), M42 (hydroxyl metabolite at the 5-position of the pyrimidinyl moiety of TMC278), and M44 (monooxygenated-TMC278) and represented 8.7%, 5.3%, and 4.3% of the dose, respectively. In addition, M30 (carboxylic acid) and M48 (unknown), which co-eluted, represented 3.1% of the dose. Other minor fecal metabolites (M23 co-eluted with M27, M37, M40, M46, and M49) were also detected and individually these metabolites did not represent more than 2% of the administered dose.

In urine, several minor metabolites (M3, M12, M14, M19, M25, M30, and M36) were identified; none of which represented more than 0.08% of the radioactive dose.

In plasma, unchanged TMC278 was the only radioactive component detected. Minor metabolites that were present in trace amounts and were detected only by LC-MS included M15 (*N*-glucuronide), M19 (glucuronide), M27, M30, and M33.

5.3.2.4. RPV: Humans

Six healthy male subjects received a single oral dose of 150 mg [^{14}C]TMC278 base (administered as a PEG400 formulation) (m2.6.5, Section 10.2.5, TMC278-NC157/TMC278-NC119). Urine and plasma were collected for up to 1 week after dosing; feces were collected for up to 2 weeks after dosing. Plasma concentrations of TMC278 were measured by LC-MS/MS. Radioactivity levels were determined by liquid scintillation counting. Metabolite profiles were determined by reversed-phase radio-HPLC and identification of the metabolites was carried out using co-chromatography with synthesized metabolites, LC-MS/MS, and NMR analysis. In plasma, the more sensitive method LC-ARC (liquid chromatography-accurate radioisotope counting) was used for metabolite profiles.

After oral administration, radioactivity was mainly excreted in feces (85% of the dose over the 14-day period), while in urine a low amount of radioactivity was excreted (6.1% of the dose over the 7-day period). Unchanged TMC278 represented on average 26% of the administered dose in feces. Unchanged TMC278 was not detected in urine.

TMC278 was extensively metabolized. The most important biotransformation pathway of TMC278 was oxidation.

The most abundant fecal metabolite was M42, which accounted on average for 16% of the dose. M33 (hydroxymethyl-TMC278) accounted for 3.0% of the administered dose, M30 (carboxylic acid derivative) for 2.7%, and a metabolite of unknown structure (M35) accounted for 2.2% of the administered dose. Some minor metabolites resulting from further biotransformation of M33 (M27, M11 and M23) were also detected (each < 1.6%).

In urine, apart from M30 (0.03%), metabolites were Phase II metabolites (glucuronides (0.9% of the administered dose) or glutathione-derived (1.2% of the administered dose) conjugates).

In plasma unchanged drug accounted for the major part of the total radioactivity. Several minor metabolites were detected, namely, the glucuronide of TMC278 (M15), the tricyclic metabolite (M27), and hydroxymethyl TMC278 (M33); others (glucuronide of hydroxymethyl TMC278, and of hydroxylated TMC278) were only present in trace amounts.

In general, all identified metabolites in human matrices were also detected in at least 1 animal species (see Figure 13).

The antiviral activities of M33 and M42 were tested on a panel of wild-type and mutant HIV-1 virus strains to determine their in vitro antiviral activity. The 50% effective concentration values for the wild-type virus were 0.4 nM for M33, 18 nM for M42, and 0.5 nM for TMC278 (m2.7.2 Section 4.1.1.3.3).

5.3.3. TAF and TFV: Metabolism In Vivo

Based on the studies from mouse, rat, dog and human (m2.6.5, Section 10.3, AD-120-2008, AD-120-2012, AD-120-2021; and GS-US-120-0109), relative quantification of TAF metabolites in plasma as percent total AUC and in urine, feces, and bile as percent total dose recovered in different species is summarized in Table 21. Endogenous purine metabolites including hypoxanthine, xanthine, allantoin, and uric acid were observed in all species. Tenofovir accounted for a majority of drug related material in plasma, urine, and feces from all species except for human plasma, in which uric acid was the predominant metabolite accounting for 73.9% of the total AUC over 96 hours. M18 was the major metabolite in rat bile accounted for 63% of total radioactivity recovered in bile. M18 and its oxidized metabolite, M16 were the major metabolites in dog bile accounted for 29 and 38% of total radioactivity recovered in bile. Various oxidative metabolites were found in dog bile. No metabolites unique to human were observed.

Tenofovir alafenamide-related metabolites were also monitored in kidney, liver, and nasal turbinate from mice (m2.6.5, Section 10.3.1, AD-120-2012). Most of the radioactivity was associated with TFV in kidney and liver and xanthine (M7) was the major identified metabolite in nasal turbinates. In dog, TAF-related metabolites were monitored in bone and liver and most of the radioactivity in these tissues was associated with TFV (m2.6.5, Section 10.3.3, AD-120-2008).

M18 (isopropylalaninyl TFV) and M28 (alaninyl TFV) are considered to be intermediate metabolites during intracellular conversion of TAF to TFV. In the metabolite profiling study in dog, M28 was not detected in this study although it has been qualitatively detected previously in dog plasma at 15 minutes post dose {23907}. It is possible that M28 may be formed transiently at low levels. M18 was detected as a minor metabolite in plasma, urine, and liver. Relatively high levels of M18 were observed in bile. Low levels of M28 were observed in rat and mouse plasma with relatively high levels in rat bile.

Table 21. Relative quantification of TAF Metacolites in Plasma, Urine, Feces, and Bile as % Total Dose

		Plasma[a]	Urine	Feces	Bile
Mouse	TFV (M12)	54.8	18.1	30.7	NA[b]
	M28	1.02	0	0.7	NA
	Allantoin (M27A)	12.2	2.6	0.4	NA
	Uric acid (M27B)	19.4	0	0	NA
	Unknown metabolites	12.3	3.2	0	NA
	Total	100	23.9	31.8	NA
Rat	TFV (M12)	66.7	17.1	63.6	NA
	M28	5.8	0	0	NA
	Allantoin (M27A)	23.2	0.1	0	NA
	Unknown metabolites	4.3	1.6	0	NA
	Total	100	18.8	63.3	NA
Rat (BDC[c])	TFV (M12)	NA	17.1	61.7	0.66
	M28	NA	0.4	0	1.17
	Allantoin (M27A)	NA	0.2	0	0
	Uric acid (M27B)	NA	0	0	0.02
	Unknown metabolites	NA	1.7	0	0
	Total	NA	19.4	61.7	1.85
Dog	TAF	1.3	1.3	0	NA
	TFV (M12)	68.3	24.2	20.8	NA
	M11	0	0.4	0.4	NA
	M17	0.44	0	0	NA
	M18	17.6	0.2	0	NA
	M20	0.2	0	0	NA
	Unknown metabolites	12.2	3.0	0.2	NA
	Total	100	29.1	21.4	NA

		Plasma[a]	Urine	Feces	Bile
Dog (BDC)	TAF	NA	1.3	0	0.2
	TFV (M12)	NA	16.8	26.4	1.0
	M11	NA	0.4	0.7	0.1
	M14	NA	0	0	0.2
	M16	NA	0	0	4.4
	M17	NA	0	0	0.5
	M18	NA	0	0	3.4
	M19	NA	0	0	0.1
	M21	NA	0	0	0.4
	M22	NA	0	0	0.2
	M23	NA	0.2	0	0.2
	Unknown metabolites	NA	2.9	0	0.9
	Total	NA	21.6	27.1	11.6
Human	TAF	1.8	1.41	0	NA
	TFV (M12)	1.5	22.2	31.4	NA
	Uric acid (M27B)	73.9	1.93	0	NA
	Xanthine/hypoxantine (M7.M8)	0	0.26	0	NA
	Unknown metabolites	22.8	0	0.29	NA
	Total	100	25.8	31.7	NA

a Plasma data represent % of total AUC.
b NA = not applicable
c BDC = bile duct cannulated

5.3.3.1. TFV: Monkeys

The kinetics of intracellular TFV anabolism in PBMCs, red blood cells (RBCs), and lymph nodes were studied in monkeys that received a single dose of either 15, 30, or 60 mg/kg of [^{14}C]TFV subcutaneously (m2.7.2, Section 4.1.1.1.1.1, [P2001025]). Tenofovir was efficiently taken up by PBMCs and anabolized to TFV-DP, with intracellular concentrations of the active antiviral anabolite reaching 1.6 μM (60 mg/kg dose group). The half-life of TFV-DP in this experiment was > 50 hours. Similar concentrations of TFV anabolites were observed in RBCs. Significant intracellular concentrations of TFV and its anabolites were observed in axillary, inguinal, and mesenteric lymph nodes. This long intracellular half-life of the active diphosphate form observed both in vitro and in vivo supports the proposed once daily clinical dosing regimen.

5.4. FTC/RPV/TAF: Metabolism

While RPV is primarily metabolized by CYP3A, TAF and FTC do not interact with drug metabolizing enzymes as inhibitors or inducers at clinically relevant concentrations. Therefore, metabolic drug interactions between these agents are unlikely.

Emtricitabine and TAF are analogs of 2 different nucleosides, cytidine and adenosine, respectively, and do not share a common intracellular metabolism pathway for pharmacological activation through phosphorylation. In experiments where both drugs were incubated together at concentrations higher than achieved in the plasma, the intracellular activation of TFV to its active diphosphate was not negatively influenced by the presence of FTC, and the activation of FTC to FTC-triphosphate, was not negatively affected by the presence of TFV (m2.7.2, Section 4.1.1.7.2, [PC-164-2002]). Also, because the 2 drugs form analogs of different nucleotides, there should be no competition for incorporation by HIV-1 RT and subsequent chain termination. This was confirmed in vitro in antiviral assays where strong synergy between the 2 compounds was observed (m2.7.2, Section 4.1.3.3 [PC-183-2004]). Similarly, because of the highly restricted substrate specificity of the enzymes catalyzing the phosphorylation of FTC and TFV, inhibition of pharmacological activation by RPV is unlikely and, again, there is no evidence for antagonism in antiviral assays in vitro.

6. EXCRETION

6.1. Route and Extent of Excretion

6.1.1. FTC

6.1.1.1. Excretion of Radioactivity after Administration of [^{3}H]FTC to Mice

Male CD-1 mice were dosed orally with 120 mg/kg [^{3}H]FTC (m2.6.5, Section 10.1.1, TEIN/93/0015). Recovery of radioactivity up to 72 hours postdose totaled 85.0% ± 4.2% of the dose, with 66.8% ± 7.0% in urine and 18.1% ± 3.1% in feces. The majority of the radioactivity was excreted during the first collection period (0–24 hours postdose), with 62.3% ± 7.6% of the dose in urine and 15.9% ± 3.0% of the dose in feces.

6.1.1.2. Excretion of Radioactivity after Administration of [^{3}H]FTC to Rats

Male Sprague-Dawley rats were dosed intravenously or orally with 10 mg/kg [^{3}H]FTC and urine and feces collected for 6 days {4570}. After IV administration, recovery of radiolabel in excreta was 96% ± 3.7%, with 91% ± 3.4% in urine and 5.0% ± 1.6% in feces. Results were similar following oral dosing, with total recovery of 99% ± 3.2% and 74% ± 2.8% in urine and 25% ± 1.6% in feces.

6.1.1.3. Excretion of Radioactivity after Administration of FTC to Monkeys

Following oral administration of 80 mg/kg [^{3}H]FTC to female cynomolgus monkeys, an average of 83.8% ± 3.8% of dosed radioactivity was recovered by 72 hours postdose, with 41.2% ± 6.4% of the dose in urine, 33.1% ± 10.0% in feces, and 9.6% ± 6.7% in cage washings (m2.6.5, Section 10.1.3, TEIN/93/0016). The majority of the radioactivity was recovered in the first collection phase (32.9% ± 8.6% in the 0–8 hour urine sample and 23.6% ± 15.9% in the 0–24 hour feces sample).

In a second study, an oral dose of 200 mg/kg [^{14}C]FTC was given to male cynomolgus monkeys and urine and feces were collected up to 120 hours postdose (m2.6.5, Section 10.1.2, TOX063). The excretion pattern was similar to the previous study: total recovery of radioactivity averaged 76.2% ± 4.1% of the dose, with 40.8% ± 8.5% of the dose in urine and 35.4% ± 8.6% in feces. However, excretion of radiolabel took longer than the previous study, with cumulative recovery of radiolabel in urine of 15.1% ± 5.1% by 12 hours postdose and 24.8% ± 7.3% by 24 hours postdose.

6.1.2. RPV

6.1.2.1.1. RPV: Mouse, Rat, Dog, and Human

The excretion of TMC278 was studied after single oral administration of [^{14}C]TMC278 base in male and female CD-1 mice at 20 and 320 mg/kg, in Sprague Dawley rats at 40 mg/kg, and in male beagle dogs at 5 mg/kg (m2.6.5, Section 10.2, TMC278-NC190, TMC278-NC113, TMC278-NC145, and TMC278-NC114). Healthy male subjects were dosed orally with 150 mg of [^{14}C]TMC278 base (m2.6.5, Section 10.2.5, TMC278-NC157). Urine and feces samples were

collected up to 96 hours after dosing in rodents; up to 168 hours after dosing in dogs and humans (only urine); and up to 336 hours in humans (only feces). Total radioactivity was measured by scintillation counting.

In rodents, the total radioactivity was rapidly excreted with 90% to 94% (at 20 mg/kg) and 69% to 74% (at 320 mg/kg) of the radioactive dose eliminated in mice, and 79% to 84% eliminated in rats within the first 24 hours after dosing. In dogs, excretion was relatively slow, with 54% of the radioactive dose eliminated within the first 24 hours. In mice, rats, and dogs, the predominant route of excretion of [^{14}C]TMC278 was via the feces. The majority of the total radioactivity was eliminated in feces as unchanged TMC278 in mice (33% to 34% at 320 mg/kg), in rats (43% to 47%), and in dogs (43%) at 48 hours after dosing. Only in mice at 20 mg/kg, 1 metabolite M42 was the most abundant in feces. Renal excretion was very limited (0.45% to 4.2% of the radioactivity dose) in all animal species and the amount of unchanged TMC278 in urine was negligible. The excretion was virtually complete at 96 hours after dosing in rodents and at 168 hours after dosing in dogs (Table 22). In a biliary excretion study in male Sprague Dawley rats, the amount of radioactivity excreted in bile within 24 hours after dosing was rather low, only 18% and 25% of the administered radioactivity, in restrained and nonrestrained animals, respectively. The amount of unchanged TMC278 excreted in bile during this time period was negligible (about 0.2%). The biliary excretion study demonstrated that the major part of unchanged TMC278 excreted in feces in rats had not been absorbed.

The excretion of TMC278 in humans was similar to that seen in the nonclinical species. Eighty-five percent of the dose was excreted in feces and excretion was virtually complete at 336 hours after dosing. Unchanged TMC278 represented on average 26% of the administered dose in feces. In humans, the amount of total radioactivity recovered in urine was somewhat higher (6.1% of the administered dose over the 7-day period) than in animals. Unchanged TMC278 in urine was negligible.

Table 22. Urinary and Fecal Excretion of the Radioactivity Following a Single Oral Dose of ^{14}C-TMC278 base in Mouse and Rat at 96 Hours After Dosing and in Dog and Human at 168 Hours After Dosing

% of Administered Dose	Mouse[a]				Rat[b]		Dog[c]	Human[d]
	20 mg/kg		320 mg/kg		40 mg/kg		5 mg/kg	150 mg
	Male	Female	Male	Female	Male	Female	Male	Male
Urine	3.51	4.19	1.84	3.62	0.45	1.77	1.73	6.13
Feces	87.8	87.1	95.8	88.8	93.3	92.6	94.7	85.1
Cage washings	3.61	3.79	1.18	3.37	0.12	0.68	0.38	—
Total Recovered	**94.9**	**95.1**	**98.9**	**95.8**	**93.9**	**95.1**	**96.8**	**91.2[e]**

a Study TMC278-TiDP6-NC190
b Study TMC278-NC113
c Study TMC278-NC114
d Study TMC278-TiDP6-NC157
e Expressed as percent of the administered dose in the 0-168h urine and 0-336h feces

6.1.3. TAF/TFV

6.1.3.1. Excretion of Radioactivity after Administration of [^{14}C]TAF to Mice

After oral administration of 100 mg/kg [^{14}C]TAF to CD-1 mice, most of the radioactivity was eliminated by 48 hours postdose (m2.6.5, Section 5.3.1, AD-120-2011). An average of approximately 61% of the radioactive dose was recovered in urine and feces from CD-1 mice through 48 hours postdose. A large amount of radioactivity (average of 6.65% of the dose) was recovered in the cage rinse. An average of 41.3 and 27.7% of the administered radioactivity were excreted in feces and urine, respectively, by 168 hours postdose. An average overall recovery of radioactivity after oral dosing to CD-1 mice was 83.2%.

6.1.3.2. Excretion of Radioactivity after Administration of [^{14}C]TAF or [^{14}C]TFV to Rats

The excretion of [^{14}C]TAF was determined after administration of a single 5-mg/kg oral dose of [^{14}C]TAF to bile duct-intact and BDC male Sprague-Dawley rats (m2.6.5, Section 5.3.2, AD-120-2020). The results from BDC rats are discussed in Section 6.2.1. Most of radioactivity derived from [^{14}C]TAF was rapidly excreted within 24 hours after oral dosing. The mean values of 71.9 and 22.2% of the administered radioactivity were excreted in feces and urine, respectively, by 168 hours postdose. The mean overall recovery of radioactivity was 96.7%.

The effect of dose on excretion of [^{14}C]TFV was evaluated in Sprague-Dawley rats following IV administration at doses of 10 or 50 mg/kg (400 µCi/kg) (m2.6.5, Section 11.3.1, 96-DDM-1278-001). Following dosing at 10 mg/kg, the mean cumulative recovery in the urine/cage wash was 85.2% ± 7.63 % by 24 hours and 92.7% ± 6.77 % by 7 days postdose. The mean terminal elimination half-life calculated from urine data was 15.82 ± 1.79 hours. The mean recovery of the administered dose in the feces was 3.18% ± 1.85% by 24 hours, and 4.48% ± 1.89% by 7 days postdose. Similar results were seen following dosing at 50 mg/kg. Tenofovir was the only species present in the urine and feces; no metabolites were detected. These results indicate that TFV is primarily excreted by renal clearance of the unchanged drug.

6.1.3.3. Excretion of Radioactivity after Administration of [^{14}C]TAF or [^{14}C]TFV to Dogs

The excretion of [^{14}C]TAF was determined after administration of a single 15-mg/kg oral dose of ^{14}C-TAF to bile duct-intact and BDC male dogs (m2.6.5, Section 12.1.1, AD-120-2007). The results from BDC dogs are discussed in Section 6.2.1. Radioactivity derived from [^{14}C]TAF was readily excreted mostly within 48 hours after oral dosing. The mean values of 37.4% and 35.9% of the administered radioactivity were excreted in feces and urine, respectively, by 168 hours postdose. Overall mean recovery of radioactivity was 80.4%.

Tenofovir excretion was also evaluated following IV administration of [^{14}C]TFV (m2.6.5, Section 12.1.2, 96-DDM-1278-002). The primary route of elimination was via the kidneys, as 70.03% of the total radioactive dose was recovered in the urine during the first 48 hours following dosing. Total fecal recovery of radioactivity was 0.42% of the total dose.

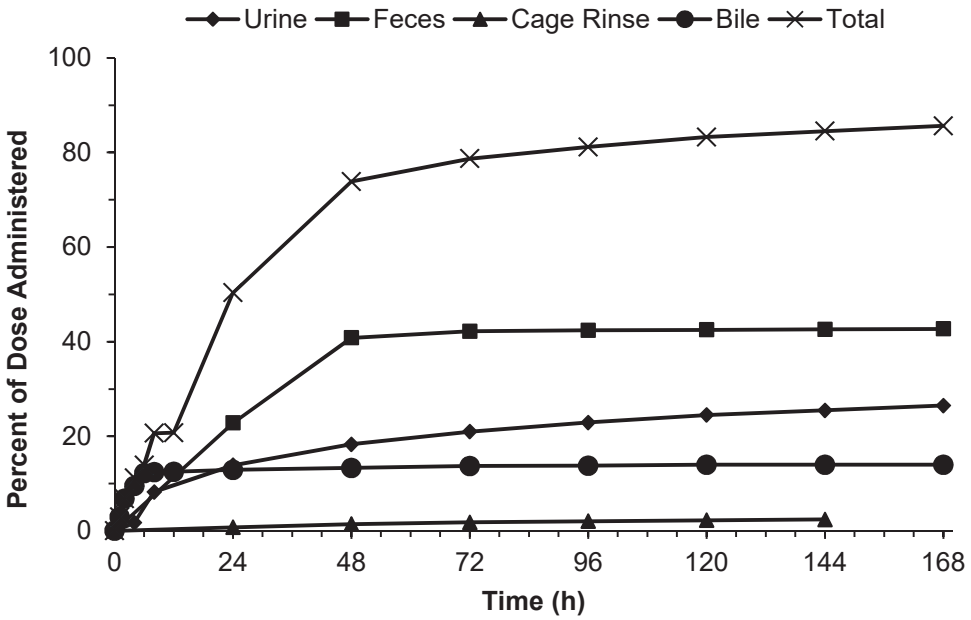
6.2. Excretion into Bile

6.2.1. TAF and TFV: Excretion into Rat and Dog Bile

The excretion of [^{14}C]TAF was determined after administration of a single 5-mg/kg oral dose of [^{14}C]TAF to BDC male Sprague-Dawley rats (m2.6.5, Section 5.3.2, AD-120-2020). Mean values of 72.6%, 23.2%, and 2.11% of the administered radioactivity were excreted in feces, urine, and bile, respectively, by 168 hours postdose. Recoveries of radioactivity in bile and urine from BDC rats indicated that at least 25% of the dose was absorbed. The mean overall recovery of radioactivity after oral dosing to BDC rats was 99.9%.

The excretion of [^{14}C]TAF was determined following oral administration of a single 15-mg/kg dose of [^{14}C]TAF to male BDC dogs (m2.6.5, Section 12.1.1, AD-120-2007). Mean values of 42.7%, 26.5%, and 14.0% of the administered radioactivity were excreted in feces, urine, and bile, respectively, through 168 hours postdose (Figure 16). Based on the radioactivity excreted in urine and bile, a minimum of approximately 41% of the orally administered dose was absorbed. The elimination of a large amount of radioactivity in bile of BDC dogs indicates that biliary excretion is a major route of elimination of [^{14}C]TAF-derived radioactivity in dogs. The overall recovery of radioactivity in BDC dogs was 86.2%. Radioactivity was measurable in urine and feces at 168 hours postdose, indicating low recoveries were probably due to radioactivity retained in the carcasses.

Figure 16. Cumulative Percent Total Radioactive Dose Recovered in Urine, Feces, Bile, and Cage Rinse Following Oral Administration of [^{14}C]TAF at 5 mg/kg to Bile Duct-Cannulated Dogs



The extent of biliary excretion of radioactivity following a single IV administration of 10 mg/kg [^{14}C]TFV to a beagle dog was evaluated (m2.6.5, Section 12.1.2, 96-DDM-1278-002). Total biliary recovery of radioactivity through 48 hours postdose was 0.26% of the total dose.

6.3. Excretion into Milk

6.3.1. TFV: Excretion into Milk

6.3.1.1. TFV: Monkeys

Milk was obtained from 2 lactating adult female rhesus monkeys following a single 30 mg/kg subcutaneous dose of TFV (m2.6.5, Section 7.3.2.2, P2000116). Both milk and serum samples were collected over time up to 24 hours postdose. Concentrations of TFV in milk reached an apparent maximum at 4 hours for 1 animal and at 1 hour in the second. Tenofovir C_{max} in milk was 4.04% and 2.02% of the observed C_{max} in plasma for the 2 animals, respectively, and declined with apparent half-lives of 10.3 and 10.9 hours. The tenofovir $AUC_{0-\infty}$ in milk was 18.6% and 21.5% of the observed $AUC_{0-\infty}$ in plasma for the 2 animals, respectively.

6.4. FTC/RPV/TAF: Excretion

Emtricitabine and TFV are almost exclusively eliminated by renal excretion. While TFV is a substrate for OAT1, OAT3, and MRP4, none of these transporters was inhibited by FTC. Rilpivirine is predominantly excreted in feces. Therefore, interactions within the components of the FTC/RPV/TAF FDC during excretion are unlikely.

7. PHARMACOKINETIC DRUG INTERACTIONS

Discussions of drug interaction liability are made by reference to current industry and US and European regulatory guidelines {11006}, {15555}, {18022}, {18670}.

7.1. FTC: Pharmacokinetic Drug Interactions

In all species examined, FTC shows high oral bioavailability, low plasma binding, and is eliminated, largely unchanged, by renal excretion. Metabolism by CYP3A (and possibly FMO) enzymes plays a minor role in FTC clearance. It is thus unlikely that FTC will be a victim of drug interactions, due to inhibition or induction of drug metabolizing enzymes or drug transporters at the intestine or liver. It is also unlikely that FTC would affect the metabolism of coadministered medications through inhibition or induction and clinical experience with FTC to date supports this conclusion. Consistent with the low induction potential, FTC did not activate human AhR or human PXR up to 50 μM in vitro (m2.6.5, Section 13.1.2, AD-162-2005).

7.2. RPV: Pharmacokinetic Drug Interactions

The in vitro interaction of TMC278 with the metabolism of sertraline (substrate of multiple CYPs, monoamine oxidase and UDP-GT), paroxetine (CYP2D6), clarithromycin (CYP3A4), sildenafil (CYP3A4), omeprazole (CYP2C19 and CYP3A4), chlorzoxazone (CYP2E1), 17α-ethinylestradiol (Phase II metabolism), S-mephenytoin (CYP2C19), and norethindrone (different isoenzymes) was investigated in a pooled batch of human liver microsomes and the same was done for abacavir (alcohol dehydrogenase) in a pooled batch of human liver cytosol (m2.6.5, Section 14.2.1, TMC278-NC194).

TMC278 seemed to have a significant inhibitory effect (IC_{50} < 5 μM) on the metabolism of clarithromycin, sildenafil, S-mephenytoin, and norethindrone and a moderate effect (5 μM < IC_{50} < 10 μM) on sertraline, paroxetine, and 17α-ethinylestradiol. Omeprazole metabolism was only poorly inhibited by TMC278, displaying an IC_{50}-value of 12 μM. TMC278 has under these conditions no measurable effect on the metabolism of abacavir or chlorzoxazone, as metabolite formation of the latter compounds was not inhibited (IC_{50} > 30 μM).

These in vitro data indicate a possible effect of TMC278 on the in vivo metabolism of clarithromycin, sildenafil, S-mephenytoin, and norethindrone; and also, albeit somewhat less likely, with sertraline, paroxetine, and 17α-ethinylestradiol. No inhibition is expected for omeprazole, abacavir, and chlorzoxazone.

In the Nonclinical Overview (Module 2.4) these results are compared to the clinical data.

The potential of RPV to inhibit renal transporters, OCT2, MATE1 and MATE2-K was assessed in vitro (m2.6.5, Section 14.2.2 and 14.2.3, TMC278-FK10042 and TMC278-FK10420). Rilpivirine showed IC_{50} values for OCT2, MATE1, and MATE2-K of 5.46, 7.51, and < 0.05 μM, respectively. Since the estimated clinical unbound plasma C_{max} for RPV is 3.5 nM, the effect of RPV on OCT2 and MATE1 is unlikely to be clinically relevant but it cannot be excluded that RPV would inhibit MATE2-K at clinically relevant concentrations. Renal clearance is, however,

negligible for RPV and it is not known what the intracellular RPV concentrations are at the local level of the MATEs' site of action. Metformin is a known substrate for OCT2 and MATEs and coadministration with RPV did not affect the plasma exposure or urine concentrations of metformin (m2.7.2, Section 3.2.3.4, [TMC278IFD1004]). OCT2, MATE1, and MATE2-K are known to be involved in renal secretion of creatinine {28345} . Small increases in serum creatinine have been observed in humans treated with RPV, likely due to inhibition of renal transporters for creatinine, but these are not considered to be clinically important {22839}.

7.3. TAF and TFV: Pharmacokinetic Drug Interactions

The pharmacokinetic profiles of TAF and TFV following oral administration of the E/C/F/TAF FDC are described in Table 23. Tenofovir alafenamide as a component of the FTC/RPV/TAF FDC is bioequivalent to E/C/F/TAF FDC (m2.7.2, Section 3.2.1.1, [GS-US-366-1159]). The unbound C_{max} of TAF was calculated based on the percent unbound value of 20% obtained from multiple human ex vivo studies which should be more clinically relevant than the value determined in vitro, which had somewhat higher percent unbound TAF.

Table 23. Steady State Pharmacokinetic Parameters for TAF and TFV

	TAF	TFV
Dose (mg)	10 (E/C/F/TAF FDC)	-
Total C_{max} (µM)	0.34	0.053
Unbound C_{max} (µM) [a]	0.068	0.053
Intestinal (µM)[b]	210	-
$C_{hep, inlet}$ [c]	1.41	

a Calculated based on percent unbound values of 20% for TAF (GS-US-120-0108 and GS-US-120-0114) and 99.3% for TFV (Section 4.1.3)
b Estimated based on TAF 25 mg dose.
c Estimated total hepatic inlet (portal vein) concentration calculated based on TAF 25 mg dose according to Obach et al. {34390}
Source: m2.7.2, Section 2.4.1.1, (TAF Population PK Report)

The potential of TAF to be a victim or perpetrator of drug-drug interactions was assessed in various in vitro systems. The potential of TAF or its metabolites to inhibit CYP enzymes and UGT1A1 or induce CYP enzymes, UGT1A1, or P-gp and serve as substrates or inhibitors of xenobiotic transporters was assessed. The effect of other drugs, including other antiviral agents that may be coadministered with TAF, on intestinal stability and the absorption potential was also determined.

7.3.1. TAF and TFV: Cytochrome P450 and UGT1A1 Inhibition

The potential for TAF and TFV to inhibit human CYP-mediated drug metabolism was examined in vitro using hepatic microsomal fractions and enzyme-selective activities (m2.6.5, Section 13.3.1, AD-120-2003 and m2.6.5, Section 13.3.2, V990172-104). The inhibitory activity of TAF with human liver microsomal CYP isozymes, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A were assessed at concentrations up to 25 µM. The inhibition constant (IC_{50}) values calculated for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and

CYP2D6 were greater than 25 µM. Tenofovir alafenamide weakly inhibited CYP3A-mediated oxidation of midazolam or testosterone with IC_{50} of 7.6 or 7.4 µM, respectively. However, the weak inhibition of CYP3A is not clinically relevant as TAF did not affect the exposure to CYP3A substrates, midazolam or rilpivirine in clinical drug-drug interaction studies (m2.7.2, Sections 2.5.2.1 and 2.5.2.2, [GS-US-120-1538 and GS-US-120-1554]). Tenofovir at 100 µM did not inhibit CYP1A2, CYP2C9, CYP2D6, CYP2E1, and CYP3A.

The potential for TAF to be a mechanism-based inhibitor of the human CYP enzymes, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A was assessed at a TAF concentration of 50 µM (m2.6.5, Section 13.3.3, AD-120-2040). There was no evidence for time- or cofactor-dependent inhibition of any enzyme by TAF and the maximum change in activity observed for any CYP was 17.4% with CYP2C8 reletive to control.

Tenofovir alafenamide was assessed for inhibition of estradiol 3-glucuronide formation in insect cell microsomal fractions containing baculovirus-expressed human UGT1A1 (m2.6.5, Section 13.3.6, AD-120-2006). Tenofovir alafenamide did not inhibit UGT1A1 up to 50 µM (IC_{50} > 50 µM).

7.3.2. TAF: Enzymology of Metabolism

Orally administered TAF undergoes intestinal absorption. During intestinal absorption, TAF may be metabolized by intestinal esterases and/or CYP enzymes. The effects of other HIV-1 PIs and CYP inhibitors on the stability of TAF in intestinal subcellular fractions were determined (m2.6.5, Section 14.3.5, AD-120-2027). Incubation of TAF with the HIV-1 PIs atazanavir or darunavir, or the CYP inhibitors, ritonavir (RTV) or COBI did not markedly affect the stability of TAF in intestinal subcellular fractions at concentrations up to 100 µM (Table 24).

Table 24. Stability of TAF in Human Intestinal Subcellular Fraction in the Absence and Presence of Test Compounds

Inhibitor	Concentration (µM)	Intestinal S9 Stability[a] $t_{½}$ (min)
Vehicle control	0	24.5 ± 4.1
Atazanavir	25	28.9 ± 5.2
	100	38.9 ± 5.3
Darunavir	25	32.2 ± 5.1
	100	30.8 ± 5.0
Ritonavir	25	19.0 ± 2.8
	100	18.9 ± 1.5
Cobicistat	25	30.1 ± 5.1
	100	32.9 ± 4.8
Dichlorvos	500	> 789[b]

a Data are mean ± SD, n = 2 (6 data points per replicate)
b Less than 10% loss of substrate in 120 minutes

To understand the enzymes involved in activation of TAF in primary human hepatocytes, cells were incubated with TAF together with known CatA inhibitors (approved hepatitis C virus NS3 inhibitors, telaprevir and boceprevir), CES1 inhibitor (bis-p-nitrophenyl phosphate, BNPP), CYP3A4 and P-gp inhibitor (COBI), or telaprevir and BNPP together (m2.6.5, Section 14.3.7, AD-120-2031). The metabolism of TAF was inhibited by BNPP in a dose-dependent manner. Little or no effect on TFV-DP formation was observed with telaprevir, boceprevir, or COBI. When telaprevir and BNPP were combined a greater inhibition was seen at higher concentrations of the 2 compounds. These results indicated that TAF is primarily hydrolyzed by CES1 and CatA also made a contribution in primary human hepatocytes. Several CES1 genetic variants that are associated with the enzyme activity have been identified at very low frequency; G143E (heterozygous: 2%-4% and homozygous: 0.05%) and D260fs (very rare) {26538}, {26712}. In a study of hepatitis B virus-infected subjects, a total of 42/51 (82.4%) patients were genotyped for the rs71647871 (G143E) variant of CES1. All patients were found to carry the reference homozygous genotype, ie, there were no carriers of the minor allele of this variant genotype. In addition, since TAF can be activated by both CES1 and CatA in liver, the genetic polymorphisms causing a marked effect on TAF activation should be extremely rare.

7.3.3. TAF: Assessment of Induction Liability

The potential for TAF to induce human drug metabolizing enzymes and drug transporters through the activation of human AhR or human PXR was evaluated in cell-based systems (m2.6.5, Section 13.3.4, AD-120-2005). For PXR activation, at 50 µM TAF the extent of activation of PXR was only 23% of the maximal effect of rifampicin and 15 µM TAF demonstrated activation of < 5% of the maximal induction elicited by rifampicin. Tenofovir alafenamide did not activate AhR up to 50 µM, the highest concentration tested. Therefore, TAF is unlikely to activate either of these human xenobiotic receptors.

The induction of CYP, P-gp, and UGT1A1 mRNA and CYP activity by TAF was assessed in cultured human hepatocytes from 3 separate donors treated with 1, 10, and 100 µM TAF added once daily for 3 consecutive days (m2.6.5, Section 13.3.5, AD-120-2032). CYP induction data as mean fold increase in mRNA levels and activity upon treatment with TAF and corresponding positive controls are summarized in Table 25. Due to cytotoxicity, the cell viability was significantly affected at 100 µM TAF and mixed responses to TAF with increased mRNA levels and reduced CYP activities were observed. At noncytotoxic concentrations of TAF (1 and 10 µM), no significant increases in the mRNA levels and the CYP activities were observed. After treatment with 10 µM TAF, the mRNA levels of CYP1A2 and CYP3A4 increased by 3.0- and 8.3-fold which correspond to 3% and 6% of the induction levels observed with the respective positive controls. Therefore, TAF showed little or no potential for CYP induction at clinically relevant concentration (1 µM). No significant induction of P-gp and UGT1A1 mRNA was observed (less than 2-fold). Furthermore, TAF is unlikely to be a clinically relevant inducer as it did not affect the exposure to midazolam or rilpivirine (m2.7.2, Sections 2.5.2.1 and 2.5.2.2, [GS-US-120-1538 and GS-US-120-1554]).

Table 25. Effect of TAF Treatment on CYP mRNA Levels and Activity in Cultured Human Hepatocytes (mean, n = 3 donors)

	Mean Fold Increase (% Positive Control)					
	mRNA			Activity[c]		
Concentration	CYP1A2	CYP2B6	CYP3A	CYP1A2	CYP2B6	CYP3A
1 µM TAF	1.2 (<1%)	0.95 (<1%)	0.92 (<1%)	1.0 (<1%)	1.1 (<1%)	0.97 (<1%)
10 µM TAF	3.0 (3%)	1.6 (4%)	8.3 (6%)	1.4 (1%)	0.85 (<1%)	0.99 (<1%)
100 µM TAF[a]	6.9 (8%)	2.5 (10%)	44 (36%)	0.84 (<1%)	0.42 (<1%)	0.37 (<1%)
Positive control[b]	72	16	120	28	13	29

a The viability of the hepatocytes was affected at this concentration of TAF and therefore caution should be taken when interpreting the corresponding induction data.
b Positive controls 50 µM omeprazole, 1000 µM phenobarbital, and 10 µM rifampin for CYP1A2, CYP2B6, and CYP3A, respectively.
c Phenacetin, bupropion, and testosterone were used as probe substrates for CYP1A2, 2B6, and 3A, respectively.

7.3.4. Potential for Transporter-Mediated Drug Interactions with TAF and TFV

The potential for TAF and TFV to inhibit or to act as substrates for drug transporters has been assessed in vitro. Inhibition constants and substrate assessments for tested transporters are presented Table 28 and Table 29 in the following section. Tenofovir alafenamide showed little or no inhibition of the transport of model substrates by P-gp, BCRP, OAT1, OAT3, and OCT2 (m2.6.5, Section 14.3.2, AD-120-2019 and m2.6.5, Section 14.3.6, AD-120-2036). Weak inhibition of OATP1B1, OATP1B3, BSEP, OCT1, and MATE1 was observed but none of these transporters was inhibited by 50% at 100 µM TAF which is approximately 200-fold over the total maximal plasma concentrations. Therefore, TAF is unlikely to be a perpetrator of transporter-mediated drug interactions.

Tenofovir alafenamide is a substrate for intestinal efflux transporters, P-gp and BCRP. An increase in TAF absorption was observed in the presence of efflux transport inhibitors, CsA or COBI in vitro (m2.6.5, Section 3.3.1, AD-120-2037 and m2.6.5, Section 14.3.3, AD-120-2013). Cobicistat is a weak inhibitor of intestinal efflux transporters, but high concentrations of COBI in the intestinal lumen, achievable briefly during absorption, may inhibit P-gp and result in increase in TAF exposure. As shown in Table 26, in the presence of 90 µM COBI in the Caco-2 bidirectional permeability assay, TAF forward permeability increased 4.6-fold and the efflux ratio significantly decreased, suggesting an efflux transporter mediated drug interaction (m2.6.5, Section 14.3.3, AD-120-2013). The effect of CsA on TAF oral bioavailability was also assessed in vivo in dogs (m2.6.5, Section 14.3.15, AD-120-2035). As described in Table 27, plasma pharmacokinetic parameters were determined in dogs following oral administration of TAF at 2 mg/kg to untreated or pretreated animals with 75 mg CsA. The CsA pretreatment increased the plasma exposure to TAF and oral bioavailability by approximately 10-fold, while the pharmacokinetic profile of TFV was slightly increased by CsA. Consistent with the increased TAF plasma exposure, the exposure to TFV-DP in PBMCs isolated from the CsA pretreated dogs was approximately 2-fold higher than that in cells from untreated animals. These results suggest that coadministration of efflux inhibitors increases TAF absorption and may potentiate the antiviral efficacy by increasing the TFV-DP levels in PBMCs.

Table 26. Effect of COBI on the Bidirectional Permeability of TAF in Caco-2 Cells

Inhibitor	Direction	TAF Initial Concentration (µM)	Recovery (%)	P_{app} ($x10^{-6}$ cm/s)	Efflux Ratio
None	Cell-Free	9.61	119	30.8	—
	Forward	9.92	64	0.74	20
	Reverse	8.71	102	15.1	
COBI (90 µM)	Forward	11.0	101	3.4	1.6
	Reverse	11.4	115	4.9	

Table 27. Mean Plasma Pharmacokinetic Parameters for TAF and TFV Following Oral Administration of TAF to Male Beagle Dogs

	No CsA Pretreatment		Pretreatment with 75 mg CsA	
Parameter	TAF	TFV	TAF	TFV
AUC_{0-t} (nM•h)	31.4	1140	312	1330
t_{max} (h)	0.14	0.667	0.24	1.0
C_{max} (nM)	109	342	813	187
$t_{½}$ (h)	0.21	>24	0.15	>24
%F	1.67	NA	16.6	NA

NA = Not applicable

Tenofovir alafenamide was found to be a substrate for hepatic uptake transporters, OATP1B1 and OATP1B3 (m2.6.5, Section 14.3.4, AD-120-2022). Exposure to TAF may be affected by inhibitors of these transporters or genetic polymorphisms that affect the transport activities. The effects of differences in OATP1B1 and OATP1B3 activity are, however, not expected to be clinically relevant given the high passive permeability of TAF. Unlike TFV, TAF was not a substrate for renal transporters, OAT1 and OAT3.

The route of elimination of TFV is renal excretion by a combination of glomerular filtration and tubular secretion. In order to understand the role of transporters in the renal secretion of TFV and to explore potential drug interactions based on these transport systems, the interactions of TFV with a variety of both uptake and efflux transporters were studied in vitro.

Results of in vitro transport studies indicate that the active tubular secretion of TFV is mediated by human OAT1 (basolateral uptake) and MRP4 (apical efflux) transporters acting in series in proximal tubules (m2.6.5, Section 14.3.14, PC-103-2001; m2.6.5, Section 14.3.8, AD-104-2001; m2.6.5, Section 14.3.9, AD-104-2002), {2520}, {7299}, {8418}. Human OAT3 may play a secondary role in the tubular uptake of TFV. Neither P-gp nor MRP2 appear to be involved in the tubular efflux of TFV. As the primary transporter handling the tubular uptake of TFV, OAT1 has been assessed for its potential role in drug interactions between TFV and other therapeutics

including antibiotics, anti-inflammatory agents, and other antivirals (including PIs). Under physiologically relevant conditions, none of the tested drugs affected OAT1-mediated transport of TFV, indicating a low potential for renal interactions with TFV due to inhibition of this pathway (m2.6.5, Section 14.3.10, PC-104-2010 and m2.6.5, Section 14.3.11, PC-104-2011), {2520}.

The results from in vitro studies investigating the contribution from MRP1 in tubular reabsorption of TFV (m2.6.5, Section 14.3.12, PC-104-2014) indicated that MRP1 is not involved in the reabsorption of TFV at the basolateral membrane of proximal tubule cells.

Tenofovir did not inhibit the activity of human OCT2 or MATE1 (IC_{50} > 300 μM) so TFV is unlikely to cause drug interactions through inhibition of these transporters (m2.6.5, Section 14.3.13, AD-104-2012).

7.4. FTC/RPV/TAF: Pharmacokinetic Drug Interactions

While RPV is primarily metabolized by CYP 3A, FTC, TAF and TFV are not clinically relevant substrates, inhibitors, or inducers. As described above (Section 5.4), TFV and FTC do not inhibit each other's pharmacological activation through phosphorylation. Thus, the drug interactions through drug metabolizing enzymes within the three compounds are unlikely. Coadministration of strong inducers or inhibitors of CYP3A may affect exposure to RPV.

In addition to the studies described for FTC and TAF, a number of transporter studies were conducted with the STB components, EVG, COBI, FTC, and TFV. Since TFV is the major circulating metabolite for both TDF and TAF, these results are relevant to evaluate the potential for transporter-mediated drug-drug interactions among these components.

Results from all the transporter substrate and inhibition studies for FTC, RPV, TAF, and TFV are summarized in Table 28 and Table 29. Emtricitabine, TAF, and TFV do not inhibit any of the transporters tested at clinically relevant concentrations in vitro. Therefore, FTC, TAF, and TFV are unlikely to be a perpetrator of transporter-mediated drug interactions.

Renal excretion of TFV is facilitated by basolateral uptake by OAT1 and OAT3 and apical efflux by the MRP4 efflux transporter. There is no evidence for inhibition of TFV renal excretion by FTC as it shows undetectable inhibition of OAT1, OAT3, and MRP4 in vitro.

While RPV was found to inhibit P-gp with IC_{50} of 9.2 μM in vitro, it is not a clinically relevant inhibitor as exposure of digoxin and TAF was not affected by RPV (m2.7.2, Sections 1.2.1 and 1.2.2, [TMC278IFD1001, GS-US-120-0117, and GS-US-120-1554]).

As discussed in Section 7.2, inhibition of OCT2 and MATE1 by RPV observed in vitro is unlikely to be clinically relevant. In addition, inhibition of MATE2-K by RPV may not result in drug-drug interactions with xenobiotics which are substrates for both MATE1 and MATE2-K. Drug interactions through inhibition of these transporters by RPV within the components of the FTC/RPV/TAF FDC are unlikely as FTC and TAF components of the FTC/RPV/TAF FDC were clinically bioequivalent to the E/C/F/TAF FDC (m2.7.2, Section 3.2.1.1, [GS-US-336-1159]).

Table 28. Transporter Substrate Assessment of FTC/RPV/TAF Components

Transporter	Substrate Assessment (y/n)				m2.6.5 Section, Report
	FTC	RPV	TAF	TFV	
P-gp	n	n	y	n	Section 14.4.3, AD-236-2004 Section 14.3.1, AD-120-2018 Section 3.2.1, TMC278-NC104
BCRP	n	ND	y	n	Section 14.4.4, AD-236-2005 Section 14.3.1, AD-120-2018
OATP1B1	ND	ND	y	ND	Section 14.3.4, AD-120-2022
OATP1B3	ND	ND	y	ND	Section 14.3.4, AD-120-2022
OAT1	n	ND	n	y	Section 14.4.8, AD-236-2010 Section 14.3.10, PC-104-2010 m2.6.3, Section 1.6, PC-120-2018
OAT3	y	ND	n	y	Section 14.4.8, AD-236-2010 Section 14.3.10, PC-103-2001 m2.6.3, Section 1.6, PC-120-2018
OCT1	ND	ND	n	n	Section 14.3.6, AD-120-2036 Section 14.3.14, PC-103-2001
OCT2	n	ND	ND	n	Section 14.4.9, AD-236-2011 Section 14.3.14, PC-103-2001
MRP1	ND	ND	ND	n	Section 14.3.12, PC-104-2014
MRP2	n	ND	ND	n	Section 14.4.11, AD-236-2013 Section 14.3.8, AD-104-2001
MRP4	ND	ND	ND	y	Section 14.3.8, AD-104-2001

BCRP = breast cancer resistance protein; MDR1 = multidrug resistance protein 1; MRP1 2, or 4 = multidrug resistance associated protein 1, 2, or 4; n = no; ND = not determined; OAT1 or 3 = organic anion transporter 1 or 3; OATP1B1 or B3 = organic anion transporting polypeptide 1B1 or B3; OCT1 or 2 = organic cation transporter 1; y = yes

Table 29. Transporter Inhibition Assessment of FTC/RPV/TAF Components

Transporter	IC_{50} (µM)				m2.6.5 Section, Report
	FTC	RPV	TAF	TFV	
P-gp	>100	9.2	>100	>1000	Section 14.4.2, AD-236-2003 Section 14.3.2, AD-120-2019 Section 3.2.1, TMC278-NC104
BCRP	>100	ND	>100	>100	Section 14.4.2, AD-236-2003 Section 14.3.2, AD-120-2019
BSEP	>100	ND	>100	>100	Section 14.4.7, AD-236-2008 Section 14.3.6, AD-120-2036
OATP1B1	>100	ND	>100	>100	Section 14.4.5, AD-236-2006 Section 14.3.2, AD-120-2019
OATP1B3	>100	ND	>100	>100	Section 14.4.5, AD-236-2006 Section 14.3.2, AD-120-2019
MATE1	>100	7.51	>100	>300	Section 14.4.1, AD-236-2001 Section 14.3.6, AD-120-2036 Section 14.3.13, AD-104-2012 Section 14.2.3, TMC278-FK10420
MATE2-K	ND	< 0.05	ND	ND	Section 14.2.3, TMC278-FK10420
OAT1	>100	ND	>100	33.8[a]	Section 14.4.6, AD-236-2007 Section 14.3.6, AD-120-2036
OAT3	>100	ND	>100	>1000	Section 14.4.6, AD-236-2007 Section 14.3.6, AD-120-2036 Section 14.3.14, PC-103-2001
OCT1	>100	ND	>100	>100	Section 14.4.7, AD-236-2008 Section 14.3.6, AD-120-2036 Section 14.3.14, PC-103-2001
OCT2	>100	5.46	>100	>300	Section 14.4.1, AD-236-2001 Section 14.3.6, AD-120-2036 Section 14.2.2, TMC278-FK10042
MRP1	ND	ND	ND	>500	Section 14.3.12, PC-104-2014
MRP2	>100	ND	ND	>100	Section 14.4.10, AD-236-2012 Section 14.3.8, AD-104-2001
MRP4	>100	ND	ND	>1000[b]	Section 14.4.6, AD-236-2007

BCRP = breast cancer resistance protein; BSEP = bile salt excretory pump; MATE = multidrug and toxin extrusion protein; MDR1 = multidrug resistance protein 1; MRP1 2, 3, or 4 = multidrug resistance associated protein 1, 2, or 4; ND = not determined; OAT1 or 3 = organic anion transporter 1 or 3; OATP1B1 or B3 = organic anion transporting polypeptide 1B1 or B3; OCT1 or 2 = organic cation transporter 1

a Binding constant for uptake into CHO cells reported by Cihlar et al, 2009 {2520}.

b Imaoka et al 2007 {10260}

8. OTHER PHARMACOKINETIC STUDIES

There are no additional studies to report.

9. DISCUSSION AND CONCLUSIONS

Emtricitabine, RPV, and TAF have been assessed individually in comprehensive nonclinical absorption, distribution, metabolism and excretion studies. Results from these studies are summarized below.

9.1. FTC

Emtricitabine is rapidly and extensively absorbed after oral administration in mice, rats, and cynomolgus monkeys, with oral bioavailability ranging from 58% to 97%. Exposure is roughly dose-proportional over most of the range explored. Therefore absorption of FTC is likely rapid and complete in humans over the clinical dose range. Emtricitabine is not extensively metabolized; therefore its bioavailability is likely to be governed only by absorption, with little or no first-pass metabolism in the intestinal wall or liver. A summary of the pharmacokinetic parameters obtained in these studies is presented in Table 30 and Table 31.

Studies of FTC in mouse, rabbit, monkey, and human plasma show that FTC has little or no measurable binding (≤ 3.6%). The observed volume of distribution for FTC (~ 0.9 L/kg) is dose-independent and close to that of total body water and suggests free distribution of FTC between tissue (intracellular and extracellular) and plasma. The low level of protein binding for FTC also suggests that drug interactions due to altered protein binding will not occur for this drug. Since FTC is largely excreted unchanged and does not bind significantly to plasma proteins, the renal clearance of FTC should be similar to its total body clearance. Emtricitabine clearance values generally exceeded the glomerular filtration rate and approach renal plasma flow, suggesting that the kidneys not only filter FTC passively, but also actively secrete it in urine, a phenomenon observed with other pyrimidine nucleosides {4570}.

Emtricitabine is widely distributed in the body. After oral administration, the highest concentrations are found in the intestine and kidneys, consistent with its absorption and elimination via these tissues. Levels in CNS tissues reach ~ 2% to 9% of those in plasma.

In radiolabel studies in mice, rats, and cynomolgus monkeys, metabolism accounts for only a minor percentage of FTC elimination. Only trace levels of metabolites were found in feces. Over 90% of the radioactivity in mouse and rat urine, and 64% of the radioactivity in monkey urine was unchanged drug. The principal metabolite was a 3'-sulfoxide (M1 or M2), accounting for approximately 2% of the dose in mice, 2.6% in rats, and 6% to 11% in monkeys. Other metabolites were detected in the urine of rats and cynomolgus monkeys, but none accounted for more than 2% of the dose. These minor metabolites may include 5-fluorocytosine, the other diastereomeric 3'-sulfoxide, and a glucuronide conjugate (M3). No 不純物FA* was detected in any samples. Thus, in contrast to other nucleoside analogues, FTC is not extensively metabolized and is eliminated primarily as unchanged drug by renal excretion. Based on these observations, it is unlikely that FTC will be subject to significant first-pass metabolism, or to changes in clearance due to hepatic disease or metabolic drug interactions.

The biotransformation of FTC in humans is similar to that in monkeys, yielding the same 3 putative metabolites (oxidation of the thiol moiety to form M1 and M2 and conjugation with glucuronic acid to form M3). These metabolites were only quantifiable at low levels in urine samples. Metabolite M2 was the most predominant, with its urinary recovery accounting for 8.7% of the dose administered. M2 was sporadically quantifiable in plasma samples of 2 out of 5 subjects and when measurable, plasma M2 concentrations were 30- to 50-fold lower than plasma FTC concentrations at corresponding time points. Urinary recoveries of metabolites M1 and M3 accounted for only 0.3% and 4% of the dose administered, respectively. These 3 metabolites along with unchanged FTC in urine accounted for essentially the entire dose recovered in urine (~86%). Emtricitabine does not inhibit human CYP and demonstrates no liability to be an inducer.

9.2. RPV

The objectives of the ADME program were to determine the pharmacokinetics of TMC278 in various animal species; to measure the exposure after oral administration; to characterize the distribution to the different organs and tissues within the body and the rates and routes of elimination; to identify the metabolites; and to predict the drug-drug interaction potential. Throughout this section, references are made to human data (where available), which are helpful in providing a clinical perspective in relation to the results obtained in nonclinical studies.

Human colon carcinoma-derived Caco-2 cells studies revealed that TMC278 can be classified as a compound with an intermediate permeability. Passive transcellular diffusion was proposed as a mechanism for TMC278 absorption. However, the solubility, and in the case of suspension and solid dosage forms, possibly also dissolution seem to limit the rate and extent of absorption. After oral administration of TMC278 base, the absolute oral bioavailability of TMC278 was 32%, 54%, 31%, and 24% in rats, rabbits, dogs, and monkeys, respectively. With citric acid in the formulation, the absolute bioavailability in dogs was increased by a factor of 2.6 and was 80% at 5 mg/kg, whereas at 80 mg/kg, there was no effect of citric acid on exposure. In rats, the bioavailability remained the same at 40 mg/kg with or without citric acid and increased with citric acid at 400 mg/kg.

After oral gavage administration of TMC278 (2 forms), peak plasma concentrations were generally reached rapidly followed by a decline at lower dose levels whereas at higher dose levels the plasma profiles showed a plateau until at least 8 hours in all species. Across the dose range studied, plasma concentrations of TMC278 increased dose-proportionally or more often less than dose-proportionally, due to poor solubility. At very high dose levels, no further increase in exposure was seen. There were no major gender differences in pharmacokinetics in mice at low dose levels and dogs, whereas in mice at high dose levels and rats, exposures in females were higher than in males (up to 2-fold in mice and 2- to 4-fold in rats). In rats, the gender effect was less pronounced after administration of the HCl salt particularly at Day 1 in the carcinogenicity study. In mice, exposure after repeated administration was comparable or slightly lower than that at Day 1 in males and females (only at 20 mg/kg/day). In females, at higher dose levels (60 to 320 mg/kg/day), exposure after repeated administration was higher (up to 2.2-fold) than at Day 1. In rats, after repeated administration of the base, systemic exposure increased slightly (up to 1.6-fold) in comparison to Day 1 in females, while in males, particularly

at high dose levels, exposure decreased slightly (up to 40%). After repeated administration of the HCl salt, the decrease in exposure seen in males was more pronounced (up to 76%) compared to base, particularly in the carcinogenicity study, while no further decrease was observed between Week 27 and Week 39. In female rats, no clear tendency for time-dependent pharmacokinetics was seen. In rabbits, the exposures obtained after repeated administration were similar to those obtained at Day 1. In dogs, after repeated administration (2 forms), exposure increased after repeated administration as compared to Day 1 mainly due to the long elimination half-life ($t_{1/2}$ = 31 hours) of TMC278. In monkeys, after repeated administration of the HCl salt, exposure had a tendency to increase, but due to the high interindividual variability it is difficult to reach firm conclusions. In the species (rats and dogs) where the base and the HCl salt were administered, the exposure was comparable at low dose in rats. At high dose in rats, exposure after administration of the base was higher (1.7- to 2.7-fold) than that after administration of the HCl salt. In dogs, no clear difference was noted. However, interindividual variability was high especially after administration of the HCl salt.

In rats, tissue distribution of [^{14}C]TMC278 and its metabolites after a single dose was rapid and extensive. The highest concentrations of radioactivity were measured in the liver, adrenal gland brown fat, and kidney. There was no evidence of undue retention and there were no indications of irreversible binding of TMC278 and its metabolites to melanin. In pregnant rats, there was distribution of [^{14}C]TMC278 to the placenta and the fetus. Total radioactivity exposure values in the placenta and in whole fetus were 0.94- and 0.64-fold those of maternal blood, respectively.

TMC278 was extensively bound to plasma proteins and this was independent of the concentration and species. In the various animal species and man, plasma protein binding ranged from 99.08% to 99.97%. TMC278 was extensively bound to human albumin and much lower to α_1-acid glycoprotein. The distribution of TMC278 to red blood cells is limited in all species.

Some differences were seen in clearance across species. In rats, blood clearance of TMC278 is moderate, whereas in rabbits, dogs, and monkeys it is low compared to the hepatic blood flow. Large differences in elimination half-life of TMC278 were observed between the rat (4.4 hours), rabbit (12 hours), dog (31 hours), and monkey (7.1 hours).Vd_{ss} is larger in rats, dogs, and monkeys and very low in rabbits.

TMC278 is metabolized by Phase I and Phase II pathways including aromatic and aliphatic hydroxylation, glutathione conjugation, N-glucuronidation, and nitrile split-off followed by reduction/oxidation, whether or not in combination with secondary pathways like glucuronidation, dehydration, and catabolism of the glutathione conjugate. In mice, oxidation of TMC278 and to a lesser extent glutathione conjugation were the predominant pathways. In rats the glutathione conjugation pathway is the predominant pathway, whereas in dog and man, oxidation of TMC278 is the predominant one. No unique human metabolites were observed. In plasma of animals and human, unchanged TMC278 was more abundant than any metabolite.

In rodents dosed with [^{14}C]TMC278, total radioactivity was rapidly excreted, whereas in dogs, excretion was relatively slow. In all animal species and human, the predominant route of excretion was via feces (> 85%) and generally, the majority of the total radioactivity eliminated was unchanged TMC278. Renal excretion of total radioactivity was very limited (0.45% to 6.1%

of the dose) in all animal species and human, and the amount of unchanged TMC278 in urine was negligible. In rats, biliary excretion was limited (18%–25% of the dose) and the amount of unchanged TMC278 in bile was negligible. In rats, there was indication that TMC278 was excreted in milk.

In vitro, the CYP3A4 isoenzyme plays a major role in the biotransformation of TMC278. TMC278 might be a very weak inducer of CYP1A2 and CYP2B6 and a moderate inducer of CYP2C19 and CYP3A4. TMC278 is an inhibitor of CYP2C8 and CYP2C9 in vitro, whereas no inhibition is expected in vivo. However, as not confirmed by subsequent studies, inhibition of CYP2E1 and CYP2C19 by TMC278 are considered not relevant.

TMC278 was shown to have P-gp inhibitor properties with an apparent IC_{50} value of 9.2 µM (3.4 µg/mL). Inhibition of transepithelial permeation of P-gp substrates cannot be excluded, but a possible effect is unlikely to be clinically relevant. P-gp inhibitors are not expected to play a role in modulating the intestinal absorption of TMC278, given the limited role of efflux transporters in the epithelial permeability of TMC278.

Ex vivo induction studies in rodents showed that TMC278 is an inducer of CYP3A-family (up to 1.7-fold in mice and up to 6-fold in rats) and CYP4A-family (up to 25-fold in mice and up to 4.7-fold in rats). Additionally, TMC278 induced UDP-GT activity in mice (up to 2.3-fold) and to a lesser extent in rats (up to 1.3-fold only at high dose in males). In dogs, treatment with TMC278 did not result in any enzyme induction. TMC278 did not affect plasma exposure to P-gp substrates including digoxin and TAF in clinical drug interaction studies (GS-US-120-0117 and GS-US-120-1554).

The recommended dose of TMC278 in HIV-infected treatment-naive patients is 25 mg once daily. At this dose level, mean C_{max} (Week 4 to 8) was 0.13 µg/mL and mean $AUC_{0\text{-}24h}$ (Week 48; population pharmacokinetics) was 2.4 µg.h/mL (m2.7.2, Sections 3.3.1.1.2 and 3.3.1.1.2, [TMC278-TiDP6-C209 and TMC278-TiDP6-C215]). These values were compared with those obtained at the highest doses tested in animal species.

At the end of administration, the C_{max} and AUC ratios (animal/human) ranged from 1.9 to 2.4 in monkeys; 25 to 42 in dogs; 97 to 115 in pregnant rabbits; 7.5 to 48 in male rats; 35 to 123 in female rats; 63 to 100 in pregnant rats; 21 to 70 in juvenile rats; and 210 to 446 in mice.

9.3. TAF

Tenofovir alafenamide generates sufficient exposure in nonclinical species chosen for assessment of toxicology. Consistent with dose-dependent permeability observed in vitro, the oral bioavailability of TAF increased with increasing dose in dogs and the observed oral bioavailability was 14.3% at the 10-mg/kg dose {23907}. Following a 15-mg/kg oral dose of [^{14}C]TAF to BDC dog, the fraction absorbed was at least 41% based on excretion in urine and bile. Therefore, hepatic extraction was calculated to be approximately 65%, which was consistent with that estimated from the in vitro stability study in dog hepatic S9 fractions (60.5%). A summary of the pharmacokinetic parameters obtained with TFV is presented in Table 32.

Following oral administration of [^{14}C]TAF to mouse, rat, and dog, [^{14}C]TAF-derived radioactivity was widely distributed to most of the tissues in all species. Consistent with high hepatic extraction, high levels of radioactivity were observed in the liver; high levels of radioactivity were also measured in the kidney. Low levels of radioactivity were observed in brain and testis in mouse. Distribution trends in the pigmented uveal tract of the eye and pigmented skin suggested that [^{14}C]TAF-related radioactivity was not selectively associated with melanin-containing tissues in the pigmented mouse. No melanin binding was observed in rats.

The biotransformation of TAF was studied in mice, rats, and dogs and compared with that in humans. Endogenous purine metabolites including hypoxanthine, xanthine, allantoin, and uric acid were observed in all species including humans. Tenofovir accounted for a majority of drug related material in plasma, urine, and feces from all species except for human plasma in which uric acid was the predominant metabolite accounting for 73.9% of the total AUC over 96 hours. No metabolites unique to human were observed. In addition, none of the intermediate metabolites formed during intracellular conversion of TAF to TFV (eg, M18 [isopropylalaninyl TFV] and M28 [alaninyl TFV]), were observed in humans. The major enzymes involved in intracellular conversion of TAF to TFV in PBMCs and primary human hepatocytes are cathepsin A and carboxylesterase 1, respectively. Tenofovir is further phosphorylated to pharmacologically active TFV-DP by cellular nucleotide kinases. These steps are usually high capacity and low affinity and are not readily inhibited by other xenobiotics.

Following oral dosing of mice, rats, and dogs with [^{14}C]TAF, the majority of radiolabel is recovered in the feces or urine in all species. The elimination of a large amount of radioactivity in bile of BDC dogs indicates that biliary excretion is a major route of elimination of [^{14}C]TAF-derived radioactivity in dogs. Total recovery of radiolabel is high for all species.

Tenofovir alafenamide is not an inhibitor of UGT1A1 and CYP enzymes except for weak inhibition observed for CYP3A in vitro. While TAF is a weak inhibitor of CYP3A in vitro, it is not a clinically meaningful inhibitor of CYP3A. Tenofovir alafenamide is not a clinically relevant inducer of CYP enzymes, UGT1A1, or P-gp. Tenofovir alafenamide is unlikely to be a perpetrator of transporter-mediated drug interactions. Since TAF is a substrate for intestinal efflux transporters P-gp and BCRP and hepatic uptake transporters OATP1B1 and OATP1B3, TAF exposure may be affected by inhibitors and/or inducers of the intestinal efflux transporters and inhibitors or genetic polymorphisms of OATPs. Tenofovir alafenamide was not a substrate for renal transporters OAT1 and OAT3 suggesting that TAF is not contributing to renal tubular cell loading of TFV; as a result, intracellular TFV concentrations in renal cells are likely to correlate with plasma TFV levels, which are lower following the administration of TAF than of TDF.

9.4. FTC/RPV/TAF

Based on the data supporting the individual components and the extensive clinical data with RPV- and FTC/TAF-containing regimens, adverse pharmacokinetic interactions that would negatively affect pharmacological efficacy are not anticipated. This is based on the well-characterized routes of elimination demonstrated for each component of the FTC/RPV/TAF FDC and the differences in physicochemical properties between the compounds, which influence drug distribution. In conclusion, the drug interactions within the components of the FTC/RPV/TAF FDC are unlikely. The pharmacokinetic assessment of FTC, RPV, and TAF supports the safe use of these agents in combination.

10. SUMMARY TABLES

Table 30. Summary of Pharmacokinetics Parameters for FTC After Single Dose Administration

Study	Species/ Strain	Dose mg/kg	Route	AUC0-∞		F	Cmax		t_{max}	MRT	MAT	t½ (minutes)		CL	Vss
				µM•h	µg •h/mL	%	µM	µg/mL	min	h	h	Absorption	Distribution/ Elimination	L/h/kg	L/kg
TEIN/93/0003	Mouse/ CD-1	10	iv	17.4	4.3*	—	—	—	—	0.38	—	—	4 / 23[a]	2.33	0.89
TEIN/93/0003	Mouse/ CD-1	10	Oral	16.7	4.1*	96	9.8	2.4*	25.4	—	—	14	16 / 137[a]	—	—
TEIN/93/0004	Mouse/ CD-1	100	iv	181	44.7*	—	—	—	—	0.42	—	ND	1.7 / 15.5 / 82[b]	2.23	0.94
TEIN/93/0004	Mouse/ CD-1	100	Oral	143	35.3*	79	89	22*	24.5	—	—	10.4	26 / 120[a]	—	—
[redacted]00101	Mouse/ CD-1	600	iv	—	473	—	—	1560	—	0.86	—	—	248	1.28	1.1
[redacted]00101	Mouse/ CD-1	600	Oral	—	296	62.7	—	139	40	2.90	2.04	—	190	—	—
TEZZ/93/0019	Monkey/ Cynomolgus	10	iv	59.9	14.8*	—	129	—	—	1.2	—	—	3.6 / 60[a]	0.7	0.8
TEZZ/93/0019	Monkey/ Cynomolgus	10	Oral	37.4	9.2*	62.7	14.1	3.5*	78	—	—	—	—	1.1	—
TEZZ/93/0019	Monkey/ Cynomolgus	80	iv	493	122*	—	1093	—	—	1.1	—	—	3.6 / 61[a]	0.7	0.8
TEZZ/93/0019	Monkey/ Cynomolgus	80	Oral	285	70.4	57.5	111	—	138	—	—	—	—	1.2	—

Study	Species/ Strain	Dose mg/kg	Route	AUC0-∞		F	Cmax		t_{max}	MRT	MAT	t½ (minutes)		CL	Vss
				µM•h	µg •h/mL	%	µM	µg/mL	min	h	h	Absorption	Distribution/ Elimination	L/h/kg	L/kg
[redacted]00301	Monkey/ Cynomolgus	80	iv	—	86.1	—	—	238	–	0.802	—	—	46.5	0.97	0.77
[redacted]00301	Monkey/ Cynomolgus	80	Oral	—	83.6	97.4		39.4	53	1.75	0.953	—	56.2	—	—
TOX063	Monkey/ Cynomolgus	200	Oral	—	133	—	—	46.7	60	—	—	—	443	1.57	—

* = µg/mL values calculated from (µM x 0.2472 = µg/mL); — = not determined / not applicable; $AUC_{0-\infty}$ = area under the plasma concentration-time curve, extrapolated from time zero to infinity; F = bioavailability; MAT = mean absorption time; MRT = mean residence time; Vdss = steady state volume of distribution
a $t_{½}\alpha$/ $t_{½}\beta$
b $t_{½2}\alpha$/ $t_{½}\beta$/ $t_{½}\gamma$
All values are mean values

Table 31. Disposition of Radioactivity after Oral Single Dose Administration of Radiolabeled FTC in Animals

Study	Species /Strain	Dose (mg/kg)/ Radiolabel	Plasma			Recovery (% dose)			Metabolites (% dose)		Urinary Metabolites (tentative identification) as % of Recovered Dose	Tissues With Highest Radioactivity Exposures
			C_{max} (µg/ml)	t_{max} (h)	$t_{½}$ (h)	Total *	Urine	Feces	Urine	Feces		
TEIN/93/0015	Mouse/ CD-1	120/^{3}H	—	—	—	85 (72 h)	66.8	18.1	9	< 1	- 64% unchanged drug - 5-fluorocytosine1.4% - two 3′-sulfoxides (1.7% and 2%) 不純物FA* not found (< LOD 0.1 %)	—
TOX092	Rat/SD	200/^{14}C	51.9	1.0	2.7	98.6	79.3 (73.3% parent drug)	18.4 (17.7% parent drug)	7	4	—	Gut, gut contents, kidney, liver, bladder (lowest in CNS)
TOX092	Rat/LE	200/^{14}C	59.2	1.0	3.4	ND	ND	ND	ND	ND	—	—
TEIN/93/ 0016	Monkey/ Cynomolgus	80/^{3}H	—	—	—	84 (72 h)	41	33	36	2	- 64% unchanged drug - 11% 3′-sulfoxide - 1.7% other metabolites (5-fluorocytosine, 2′-glucuronidated and deaminated metabolites) 不純物FA* not found, (< LOD 0.02 %)	—
TOX063	Monkey/ Cynomolgus	200/^{14}C	46.7	1.0	7.38	84.5 (120 h)	40.8 (29.2% parent drug)	35.4 (34.4% parent drug)	11.6	1.3	- 11% total metabolites - Two 3′-sulfoxides, - 2′-O-glucuronide	Mainly gut + contents; some in kidney, liver (lowest in CNS + spinal cord)

— = not determined
a Includes urine, faeces, and cage washes
All values presented are mean values

Table 32. Comparison of TFV Noncompartmental Pharmacokinetic Parameters Between Species Following Intravenous Administration of TFV

Species	Rat R2000075		Dog D2000076, 97-DDM-4331-001		Rhesus P4331-00033, P2000031		Human	
Dose (mg/kg)	**10**	**50**	**1**	**10**	**5**	**30**	**1**	**3**
N (sex)	4M	4M	4M	4M	3M/3F	3M/3F	8M	8M
C_{max} (µg/mL)	22.0	162	2.99	31.0	13.8	79.0	2.71	8.52
$AUC_{0-\infty}$ (µg·h/mL)	5.86	53.7	4.48	41.2	5.12	38.4	4.41	15.2
Terminal t½ (h)	4.02	5.41	45.3	38.6	5.37	8.79	5.27	7.80
CL (mL/h/kg)	1706	931	226	244	1031	888	236	229
C_{last} (µg/mL)	0.0309	0.0428	0.0017	0.0103	0.0042	0.0062	0.0426	0.0508
T_{last} (h)	12.0	24.0	120	120	24.0	38.0	12.0	24.0
V_{ss} (mL/kg)	2810	1120	3090	2510	1280	969	763	975

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2.6 NONCLINICAL SUMMARY

SECTION 2.6.5—PHARMACOKINETICS TABULATED SUMMARY

EMTRICITABINE/RILPIVIRINE/TENOFOVIR ALAFENAMIDE FIXED-DOSE COMBINATION (FTC/RPV/TAF FDC)

Gilead Sciences

26 May 2015

CONFIDENTIAL AND PROPRIETARY INFORMATION

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NOTE TO REVIEWER

This document contains a summary of nonclinical pharmacokinetic studies conducted in support of a fixed-dose combination (FDC) that contains the active substances emtricitabine (FTC), rilpivirine (RPV), and tenofovir alafenamide (TAF). The FDC tablet is referred to as emtricitabine/rilpivirine/tenofovir alafenamide (FTC/RPV/TAF) throughout this document.

As TAF is a new chemical entity, this nonclinical summary contains all available data on this new component while providing key data on FTC and RPV that support the efficacy and safety claims in the FDC prescribing information. Nonclinical studies have utilized TAF in most cases; however, relevant studies with the parent drug tenofovir (TFV) are also provided as applicable. Links to all study reports included in the dossier are highlighted in blue text.

In order to simplify the review, the order of presentation in each section follows the general format: FTC, RPV, and TAF (and tenofovir [TFV]). Given the lack of remarkable effects in the nonclinical studies with the individual agents, no additional pharmacokinetic studies have been conducted for the FTC/RPV/TAF combination. Some drug-drug interaction studies on FTC and TFV are presented as a componetnts of Strivild® (STB) (elvitegravir (EVG)/cobicistat (COBI)/ /FTC/TFV).

Throughout this module, TAF may be referred to by its compound number GS-7340, RPV may be referred to by its compound number TMC278, and FTC may be referred to by its compound number GS-9019.

The following conversions are also provided to aid the reviewer:

- FTC (GS-9019) 1 μM = 0.247 μg/mL
- RPV (TMC278) 1 μM = 0.366 μg/mL
- TAF (GS-7340) 1 μM = 0.477 μg/mL
- TFV (GS-1278) 1 μM = 0.287 μg/mL

1. PHARMACOKINETICS: OVERVIEW

Test Article: FTC, RPV, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Analytical Methods and Validation (FTC)					
Mouse, monkey, human plasma, and human urine	No	NA	In Vitro	[redacted], USA	97/001.01
Mouse, rabbit, monkey plasma	No	NA	In Vitro	[redacted], USA	6159v1
Mouse, rat, human plasma	No	NA	In Vitro	[redacted], USA	6447v5
Monkey, human urine	No	NA	In Vitro	[redacted], USA	7582v1
Analytical Methods and Validation (RPV)					
Mouse	No	NA	In Vitro	J&J PRD Beerse, Belgium	R278474/FK4240
Rat	No	NA	In Vitro	J&J PRD Beerse, Belgium	R278474/FK4170
Rabbit	No	NA	In Vitro	J&J PRD Beerse, Belgium	BA104
Dog	No	NA	In Vitro	J&J PRD Beerse, Belgium	R278474/FK4169
Monkey	No	NA	In Vitro	J&J PRD Beerse, Belgium	BA1062

Test Article: FTC, RPV, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Analytical Methods and Validation (TAF)					
Mouse plasma	No	NA	In Vitro	[redacted], USA	BA-120-2004
Rat plasma	No	NA	In Vitro	[redacted], USA	BA-120-2003
Rabbit plasma	No	NA	In Vitro	[redacted], USA	BA-120-2005
Dog PBMC	No	NA	In Vitro	[redacted] Canada	993680 MYS (D990175)
Dog, monkey, human plasma	No	NA	In Vitro	[redacted], Canada	TOX-120-002 Appendix 32
Monkey plasma	No	NA	In Vitro	[redacted] Canada	BA-120-2010
Monkey plasma	No	NA	In Vitro	[redacted] Canada	BA-120-2011
Monkey PBMC	No	NA	In Vitro	[redacted] Canada	BA-120-2012
Monkey PBMC	No	NA	In Vitro	[redacted] Canada	BA-120-2013
Monkey plasma	No	NA	In Vitro	[redacted], Canada	010520/PDW
Monkey plasma	No	NA	In Vitro	[redacted], Canada	010521/PHZ
Monkey PBMC	No	NA	In Vitro	[redacted], Canada	AA01240-RQZ (P2000114)

Test Article: FTC, RPV, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Analytical Methods and Validation (TFV)					
Mouse plasma	No	NA	In Vitro	[redacted], USA	P4331-00008 (97-TOX-4331-08, OLI-RE748-9807-DNS-1)
Rat, monkey plasma	No	NA	In Vitro	[redacted], USA	P1278-00001 (OLI-VRA144.1)
Rat milk	No	NA	In Vitro	[redacted], Canada	P1278-00034 (003105/OUI)
Rat plasma	No	NA	In Vitro	[redacted] Canada / [redacted] Canada	P1278-00028 (001097/NDK)
Rat plasma	No	NA	In Vitro	[redacted] Canada	001092/NGE
Dog plasma	No	NA	In Vitro	[redacted], USA	P1278-00017 (OLI-VRA144.2)
Dog plasma	No	NA	In Vitro	[redacted], Canada	P4331-0037 (003296/OTN)
Monkey plasma	No	NA	In Vitro	[redacted], Canada	P1278-00029 (002092/OFH)
Absorption After a Single Dose (FTC)					
Absorption, Bioavailability	Yes	Mouse	IV, Oral	[redacted] USA	TEIN/93/0003
Absorption, Bioavailability	Yes	Mouse	IV, Oral	[redacted] USA	TEIN/93/0004

Test Article: FTC, RPV, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Absorption, Bioavailability	Yes	Mouse	IV, Oral	[redacted] Scotland	[redacted]00101
Absorption, Bioavailability	Yes	Cynomolgus Monkey	IV, Oral	[redacted], USA	TEZZ/93/0019
Absorption, Bioavailability	No	Cynomolgus Monkey	IV, Oral	[redacted], Scotland	[redacted]00301
Absorption After a Single Dose (RPV)					
Drug Permeability and Transport	No	In Vitro	In Vitro	J&J PRD Beerse, Belgium	R278474-JRF FK4155
Drug Permeability and Transport	No	In Vitro	In Vitro	J&J PRD Beerse, Belgium	TMC278-NC104
Absorption	Yes	Mouse	Oral	J&J PRD Beerse, Belgium	TMC278-FK4259
Absorption	No	Rat	IV, Oral	J&J PRD Beerse, Belgium	TMC278-FK4195
Absorption	No	Rat	Oral	J&J PRD Beerse, Belgium	TMC278-NC106
Absorption	No	Rat	Oral	J&J PRD Beerse, Belgium	TMC278-FK4278
Absorption	No	Rabbit	IV	J&J PRD Beerse, Belgium	TMC278-FK4293
Absorption	No	Dog	IV, Oral	J&J PRD Beerse, Belgium	TMC278-FK4231

Test Article: FTC, RPV, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Absorption	No	Dog	Oral	J&J PRD Beerse, Belgium	TMC278-NC163
Absorption	No	Monley	IV, Oral	J&J PRD Beerse, Belgium	TMC278-DM02403
Absorption After a Single Dose (TAF)					
Permeability across Caco-2 cell monolayers	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2037
Absorption	Yes	Mouse	Oral	[redacted], USA	AD-120-2014
Absorption	No	Mouse	Oral	[redacted], USA	AD-120-2016
Absorption	Yes	Rat	Oral	[redacted], USA	AD-120-2015
Absorption	No	Rat	Oral	Gilead Sciences, Inc., Boulder, CO, USA (In-life phase); Gilead Sciences, Inc., Foster City, CA, USA (Analysis)	R990130
Formulation comparison	No	Rat	Oral	Gilead Sciences, Inc., Boulder, CO, USA (in-life phase); [redacted] Canada (Analysis)	R2000065
Absorption, Bioavailability	No	Dog	IV, Oral	[redacted] USA (In-life phase), Gilead Sciences, Inc., Foster City, CA, USA (Analysis)	99-DDM-1278-001-PK

Test Article: FTC, RPV, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Absorption	No	Dog	Oral	[redacted], USA (In-life phase), Gilead Sciences, Inc., Foster City, CA, USA (Analysis)	AD-120-2034
Absorption, Bioavailability	No	Rhesus Monkey	Oral	[redacted], USA, (In-life phase), [redacted] Canada (Analysis)	P2000087
Absorption, Bioavailability (TFV)	No	Rhesus Monkey	Oral, IV	[redacted] USA, (In-life phase), [redacted] Canada (Analysis)	P2000031 (P4331-00033)
Absorption After Repeated Dose (FTC)					
Toxicokinetics	Yes	Mouse	Oral	[redacted], USA Gilead Sciences Inc., Foster City, CA, USA	TOX-109
Toxicokinetics	No	Mouse	Oral	[redacted] Scotland	[redacted]00701
Toxicokinetics	No	Mouse	Oral	[redacted], USA	TOX 599
Toxicokinetics	No	Mouse	Oral	[redacted] Scotland	TOX 022
Toxicokinetics	No	Mouse	Oral	[redacted] USA	TOX 628
Toxicokinetics	Yes	Rat	Oral	[redacted] Gilead Sciences Inc., Foster City, CA, USA	TOX 108

Test Article: FTC, RPV, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Toxicokinetics	No	Rat	Oral	[redacted] Gilead Sciences Inc., Foster City, CA, USA	TOX 097
Toxicokinetics	No	Cynomolgus Monkey	Oral	[redacted], USA	TOX 600
Toxicokinetics	No	Cynomolgus Monkey	Oral	[redacted], USA	TOX 627
Toxicokinetics	No	Cynomolgus Monkey	Oral	[redacted], USA	TOX 032
Absorption After Repeated Dose (RPV)					
Toxicokinetics	No	Mouse	Oral	J&J PRD Beerse, Belgium	TMC278-NC118
Toxicokinetics	Yes	Mouse	Oral	J&J PRD Beerse, Belgium	TMC278-NC121
Toxicokinetics	Yes	Mouse	Oral	J&J PRD Beerse, Belgium	TMC278-NC119
Toxicokinetics	Yes	Mouse	Oral	[redacted], UK	TMC278-NC120
Toxicokinetics	No	Rat	Oral	J&J PRD Beerse, Belgium	TMC278-FK4103
Toxicokinetics	Yes	Rat	Oral	J&J PRD Beerse, Belgium	TMC278-FK4243
Toxicokinetics	No	Rat	Oral	J&J PRD Beerse, Belgium	TMC278-NC136

Test Article: FTC, RPV, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Toxicokinetics	No	Rat	Oral	J&J PRD Beerse, Belgium	TMC278-NC177
Toxicokinetics	Yes	Rat	Oral	[redacted], France	TMC278-TOX5692
Toxicokinetics	Yes	Rat	Oral	J&J PRD Beerse, Belgium	TMC278-NC117
Toxicokinetics	Yes	Rat	Oral	J&J PRD Beerse, Belgium	TMC278-NC101
Toxicokinetics	Yes	Rat	Oral	[redacted], UK	TMC278-NC123
Toxicokinetics	Yes	Juvenile rat	Oral	[redacted], UK	TMC278-NC168
Toxicokinetics	No	Rabbit	Oral	J&J PRD Beerse, Belgium	TMC278-NC126
Toxicokinetics	No	Dog	Oral	J&J PRD Beerse, Belgium	TMC278-FK4102
Toxicokinetics	Yes	Dog	Oral	J&J PRD Beerse, Belgium	TMC278-FK4244
Toxicokinetics	Yes	Dog	Oral	J&J PRD Beerse, Belgium	TMC278-TOX5650
Toxicokinetics	Yes	Dog	Oral	J&J PRD Beerse, Belgium	TMC278-NC116
Toxicokinetics	Yes	Dog	Oral	J&J PRD Beerse, Belgium	TMC278-NC115

Test Article: FTC, RPV, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Toxicokinetics	Yes	Dog	Oral	[redacted] France	TMC278-NC107
Absorption, Repeat Dose	No	Monkey	Oral	[redacted] Germany	TMC278-NC249
Absorption, Repeat Dose	No	Monkey	Oral	[redacted], Germany	TMC278-NC326
Absorption, Repeat Dose	No	Monkey	Oral	[redacted], Germany	TMC278-NC248
Absorption After Repeated Dose (TAF)					
Repeated dose pharmacokinetics	No	Dog	Oral	[redacted], USA (In-life phase) Gilead Sciences, Inc., Foster City, CA, USA (Analysis)	AD-120-2033
Toxicokinetics	Yes	Dog	Oral	[redacted] Canada (In-life phase), [redacted], USA (Analysis); [redacted], USA (Analysis); [redacted], Canada (Analysis)	D990175-PK
Toxicokinetics	Yes	Monkey	Oral	[redacted], (In-life phase), [redacted], Canada (Analysis)	P2000114-PK

Test Article: FTC, RPV, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Distribution (FTC)					
Plasma protein binding	No	In Vitro	In Vitro	[redacted] USA	TBZZ/93/0025
Tissue distribution, Excretion	No	Rat	Oral	[redacted] USA	TOX092
Distribution (RPV)					
Protein Binding Blood Distribution	No	In Vitro	In Vitro	J&J PRD Beerse, Belgium	TMC278-FK4217
Protein Binding Blood Distribution	No	In Vitro	In Vitro	J&J PRD Beerse, Belgium	TMC278-NC112
Protein Binding Blood Distribution	No	In Vitro	In Vitro	J&J PRD Beerse, Belgium	TMC278-NC332
Tissue Distribution	No	Rat	Oral	J&J PRD Beerse, Belgium	TMC278-NC108
Tissue Distribution	No	Pregnant rat	Oral	J&J PRD Beerse, Belgium	TMC278-NC109
Distribution (TAF)					
Plasma protein binding in vitro	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2026
Plasma protein binding in vitro of TFV	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	P0504-00039.1
Tissue distribution of radioactivity	No	Mouse	Oral	[redacted], USA	AD-120-2011

Test Article: FTC, RPV, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Tissue distribution of radioactivity	No	Rat	Oral	[redacted], USA	AD-120-2020
Absorption	No	Dog	Oral	[redacted], USA	AD-120-2009
Single dose tissue distribution	Yes	Dog	Oral	[redacted], USA	D990173-BP
Studies in pregnant or nursing animals (FTC, RPV, and TFV)					
Repeat-dose tissue distribution	No	Pregnant Mouse and Fetus	Oral	[redacted] USA	TOX103 Report Addendum
Repeat-dose tissue toxicokinetics on embryo/fetus	No	Rabbit	Oral	[redacted] USA	TOX038 Report Addendum
Absorption, Repeat Dose	Yes	Pregnant rat	Oral	J&J PRD Beerse, Belgium	TMC278-NC105
Absorption, Repeat Dose	No	Pregnant rabbit	Oral	J&J PRD Beerse, Belgium	TMC278-NC128
Absorption, Repeat Dose	Yes	Pregnant rabbit	Oral	J&J PRD Beerse, Belgium	TMC278-NC130
Single/repeat dose tissue distribution to evaluate placental transfer of TFV	No	Pregnant Rhesus Monkey and Fetus	Subcutaneous	[redacted] USA (In-life phase), Gilead Sciences, Foster City, CA, USA (Analysis)	96-DDM-1278-005
Single dose tissue distribution of TFV	No	Lactating Rhesus Monkey	Subcutaneous	[redacted], USA, [redacted] Canada (Analysis)	P2000116

Test Article: FTC, RPV, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Metabolism (FTC)					
Metabolic reaction phenotyping	No	In Vitro	In Vitro	[redacted], Canada	15396V1 (48170, PDM-007)
Metabolism, excretion	Yes	Mouse	Oral	[redacted] USA	TEIN/93/0015
Metabolism, excretion	Yes	Cynomolgus Monkey	Oral	[redacted] USA	TEIN/93/0016
Metabolism, excretion	No	Cynomolgus Monkey	Oral	[redacted] USA	TOX063
Metabolism (RPV)					
Metabolism, excretion	No	Mouse	Oral	J&J PRD Beerse, Belgium	TMC278-NC190
Metabolism, excretion	No	Rat	Oral	J&J PRD Beerse, Belgium	TMC278-NC113
Metabolism, excretion	No	Dog	Oral	J&J PRD Beerse, Belgium	TMC278-NC114
Metabolism, excretion	No	Human	Oral	J&J PRD Beerse, Belgium	TMC278-NC157
Metabolism, excretion in bile	No	Rat	Oral	J&J PRD Beerse, Belgium	TMC278-NC145
Metabolism	No	Rat	Oral	J&J PRD Beerse, Belgium	TMC278-NC290

Test Article: FTC, RPV, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Metabolism	No	Mouse, rat, rabbit, dog, human	Oral	J&J PRD Beerse, Belgium	TMC278-NC155
Metabolism	No	Mouse, rat, rabbit, dog, human	In Vitro	J&J PRD Beerse, Belgium	TMC278-FK4152
Metabolism	No	Mouse, rat, rabbit, dog, human	In Vitro	J&J PRD Beerse, Belgium	TMC278-NC102
Metabolism	No	Guinea pig, monkey	In Vitro	J&J PRD Beerse, Belgium	TMC278-NC333
Metabolism	No	Human	In Vitro	J&J PRD Beerse, Belgium	TMC278-FK4151
Metabolism	No	Human	In Vitro	J&J PRD Beerse, Belgium	TMC278-NC141
Metabolism	No	Human	In Vitro	J&J PRD Beerse, Belgium	TMC278-FK4789
Induction	No	Human	In Vitro	J&J PRD Beerse, Belgium	TMC278-NC186
Induction	No	Human	In Vitro	J&J PRD Beerse, Belgium	TMC278-FK4824
Metabolism, induction/inhibition	Yes	Mouse	Ex Vivo	[redacted], UK	TMC278-NC192
Metabolism, induction/inhibition	Yes	Rat	Ex Vivo	[redacted], UK	TMC278-NC193

Test Article: FTC, RPV, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Metabolism, induction/inhibition	Yes	Dog	Ex Vivo	[redacted], UK	TMC278-NC140
Induction/inhibition	No	Rat	Ex Vivo	J&J PRD Beerse, Belgium	TMC278-FK4247
Inhibition	No	Human	In Vitro	J&J PRD Beerse, Belgium	TMC278-FK4123
Inhibition	No	Human	In Vitro	J&J PRD Beerse, Belgium	TMC278-NC283
Effect Adrenal Gland	No	Dog	In Vitro	J&J PRD Beerse, Belgium	TMC278-FK4790
Metabolism (TAF)					
In vitro metabolism, plasma stability	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2025
In vitro metabolism, hepatic S9 stability	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2023
In vitro metabolism, intestinal S9 stability	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2024
In Vitro cytochrome P450 phenotyping	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2004
Metabolism in vitro	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2017
Metabolism in vitro of TFV	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	96-DDM-1278-003

Test Article: FTC, RPV, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Metabolite identification, mouse	No	Mouse	Oral	[redacted] USA	AD-120-2012
Metabolite identification, rat	No	Rat	Oral	[redacted] USA	AD-120-2021
Metabolite identification, dog	No	Dog	Oral	[redacted], USA	AD-120-2008
Excretion (TAF)					
Absorption and excretion	No	Dog	Oral	[redacted], USA	AD-120-2007
Absorption and excretion of TFV	No	Rat	IV	Gilead Sciences, Inc., Foster City, CA, USA	96-DDM-1278-001
Absorption and excretion of TFV	No	Dog	IV	[redacted], USA	96-DDM-1278-002
Pharmacokinetic Drug Interactions (FTC)					
Cytochrome P450 and UDP glucuronosyl transferase inhibition potential	No	In Vitro	In Vitro	[redacted], Canada	15247 (48171, PDM-006)
Induction potential of metabolizing enzymes	No	In Vitro	In Vitro	[redacted], USA	AD-162-2005
Pharmacokinetic Drug Interactions (RPV)					
Drug-drug Interactions	No	Human	In Vitro	J&J PRD Beerse, Belgium	TMC278-NC194
Inhibition of human OCT2	No	Human	In Vitro	J&J PRD Beerse, Belgium	TMC278-FK10042
Inhibition of human MATE-1 and MATE-2K	No	Human	In Vitro	J&J PRD Beerse, Belgium	TMC278-FK10420

Test Article: FTC, RPV, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Pharmacokinetic Drug Interactions (TAF)					
In vitro cytochrome P450 inhibition	No	In Vitro	In Vitro	[redacted], UK	AD-120-2003
In vitro cytochrome P450 inhibition of TFV	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	V990172-104
In vitro cytochrome P450 mechanism-based inhibition	No	In Vitro	In Vitro	[redacted], UK	AD-120-2040
In Vitro cytochrome P450 induction	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2005
Human UGT1A1 inhibition potential	No	In Vitro	In Vitro	[redacted], UK	AD-120-2006
Permeability in P-gp and BCRP overexpressing cells	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2018
Inhibition of human OATP1B1, OATP1B3, P-gp, and BCRP	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2019
Permeability across Caco-2 cell monolayers	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2013
Effect on uptake in human OATP1B1 and OATP1B3	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2022
Effect of pharmacoenhancer on intestinal stability	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2027
Inhibition of human OAT1, OAT3, OCT1, OCT2. MATE1 and BSEP	No	In Vitro	In Vitro	[redacted] Hungary	AD-120-2036
Effect of cathepsin A, carboxylesterase 1, CYP3A4 on primary human hepatocytes	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2031

Test Article: FTC, RPV, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Human hepatocyte induction potential	No	In Vitro	In Vitro	[redacted], USA	AD-120-2032
Effect of TFV on human OAT3, OCT1, and OCT2	No	In Vitro	In Vitro	[redacted] USA / Gilead Sciences, Inc., Foster City, CA, USA	PC-103-2001
Transport of TFV by MRP2 and MRP4	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-104-2001
Assessment of interaction potential between TFV and human P-gp	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-104-2002
Drug interaction with human OAT1 transport of TFV	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	PC-104-2010
Drug interaction with human OAT3 transport of TFV	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	PC-104-2011
Assessment of interaction potential between TFV and human MRP1	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	PC-104-2014
Effects of TFV on human OCT2 and MATE1	No	In Vitro	In Vitro	[redacted], USA/ [redacted], Hungary	AD-104-2012
Effect of CsA pretreatment on pharmacokinetics	No	Dog	IV, Oral	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2035
Pharmacokinetic Drug Interactions (EVG/COBI/FTC/TFV)					
Human OCT2 and MATE1 inhibition potential	No	In Vitro	In Vitro	[redacted] Hungary	AD-236-2001
Human P-gp and BCRP inhibition potential	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-236-2003

Test Article: FTC, RPV, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Permeability in P-gp overexpressing cells	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-236-2004
Permeability in BCRP overexpressing cells	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-236-2005
Inhibition of human OATP1B1 and OATP1B3	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-236-2006
Inhibition of human OAT1, OAT3, and MRP4 transporters	No	In Vitro	In Vitro	[redacted] Hungary	AD-236-2007
Inhibition of human OCT1 and BSEP transporters	No	In Vitro	In Vitro	[redacted] Hungary	AD-236-2008
Interaction of emtricitabine with human OAT1 and OAT3	No	In Vitro	In Vitro	[redacted] Hungary	AD-236-2010
Interaction of emtricitabine and tenofovir with human OCT1	No	In Vitro	In Vitro	[redacted] Hungary	AD-236-2011
Inhibition of elvitegravir and emtricitabine with human MRP2	No	In Vitro	In Vitro	[redacted] Hungary	AD-236-2012
Interaction of emtricitabine with human MRP2	No	In Vitro	In Vitro	[redacted] Hungary	AD-236-2013

Other Pharmacokinetic Studies: None

BCRP = breast cancer resistance protein; BSEP = bile salt export protein; COBI = cobicistat; CsA = cyclosporin A; CYP = cytochrome P450; EVG = elvitegravir; FTC = emtricitabine; MATE = multidrug and toxin extrusion protein; MRP = multidrug resistance-associated protein; MDR1 = P-glycoprotein (P-gp, ABCB1); OCTN1 = organic cation transporter N1; OAT = organic anion transporter; OATP = organic anion transporting polypeptide; OCT = organic cation transporter; PBMC = peripheral blood mononuclear cell; P-gp = human P-glycoprotein; PXR = pregnane X receptor; RPV = rilpivirine; TAF = tenofovir alafenamide; TFV = tenofovir; UDP = uridine diphosphate; UGT = uridine diphosphate glucuronosyl transferase

a An entry of "Yes" indicates that the study includes a Good Laboratory Practice (GLP) compliance statement.

2. PHARMACOKINETICS: ANALYTICAL METHODS AND VALIDATION REPORTS

2.1. FTC: Analytical Methods and Validation Reports

Test Article: FTC

Type of Study:	**Analytical Method Validation**		
Study No.	**Matrices**	**Analyte**	**Analytical Method**
97/001.01	Mouse, Monkey, Human Plasma and Human Urine	FTC	LC/MS (SIM)
6159v1	Mouse, Rabbit, Monkey Plasma	FTC	LC/MS/MS
6447v5	Mouse, Rat, Human Plasma	FTC	LC/MS/MS
7582v1	Monkey, Human Urine	FTC	LC/MS/MS

FTC = emtricitabine; LC/MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry

2.2. RPV: Analytical Methods and Validation Reports

Test Article: RPV

Type of Study:	**Analytical Method Validation**		
Study No.	**Matrices**	**Analyte**	**Analytical Method**
TMC278-FK4240	Mouse Plasma	RPV	LC/MS/MS
TMC278-FK4170	Rat Plasma	RPV	LC/MS/MS
TMC278-BA104	Rabbit Plasma	RPV	LC/MS/MS
TMC278-FK4169	Dog Plasma	RPV	LC/MS/MS
TMC278-NC273	Monkey Plasma	RPV	LC/MS/MS

2.3. TAF: Analytical Methods and Validation Reports

Test Article: TAF, TFV

Type of Study:	**Analytical Method Validation**		
Study No.	**Matrices**	**Analyte**	**Analytical Method**
001092/NGE	Rat Plasma	TFV	LC/MS/MS
R-BA-Tox-120-001	Rat Plasma	TFV	LC/MS/MS
BA-120-2004	Mouse Plasma	TAF, TFV	LC/MS/MS
BA-120-2003	Rat Plasma	TAF, TFV	LC/MS/MS
BA-120-2005	Rabbit Plasma	TAF, TFV	LC/MS/MS
993680 MYS	Dog PBMC	TFV	LC/MS/MS
TOX-120-002 Appendix 32	Dog, Monkey, Human Plasma	TAF, TFV	LC/MS/MS
BA-120-2010	Monkey Plasma	TAF	LC/MS/MS
BA-120-2011	Monkey Plasma	TFV	LC/MS/MS
BA-120-2012	Monkey PBMC	TFV	LC/MS/MS
BA-120-2013	Monkey PBMC	TFV	LC/MS/MS
010520/PDW	Monkey Plasma	TFV	LC/MS/MS
010521/PHZ	Monkey Plasma	TAF	LC/MS/MS
AA01240-RQZ	Monkey PBMC	TFV	LC/MS/MS

LC/MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; PBMC = peripheral blood mononuclear cell; TAF = tenofovir alafenamide; TFV = tenofovir

2.4. TFV: Analytical Methods and Validation Reports

Test Article: TFV

Type of Study:	**Analytical Method Validation**		
Study No.	**Matrices**	**Analyte**	**Analytical Method**
P4331-00008	Mouse Plasma	TFV	LC/Fluorescence
P1278-00001	Rat, Monkey Plasma	TFV	LC/Fluorescence
P1278-00028	Rat Plasma	TFV	LC/MS/MS
P1278-00034	Rat Milk	TFV	LC/MS/MS
P4331-035-3	Rabbit Plasma	TFV	LC/Fluorescence
P1278-00017	Dog Plasma	TFV	LC/Fluorescence
P4331-0037	Dog Plasma	TFV	LC/MS/MS
P1278-00029	Monkey Plasma	TFV	LC/MS/MS

LC/MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; TFV = tenofovir

3. PHARMACOKINETICS: ABSORPTION AFTER A SINGLE DOSE

3.1. FTC

3.1.1. TEIN/93/0003: Pharmacokinetics of FTC in Mice (10 mg/kg)

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics of 524W91 in Male CD-1 Mice Following Oral and Intravenous Administration	Absorption, bioavailability	FTC	TEIN/93/0003

Species	Mouse / CD-1
Feeding Condition	Not fasted
Vehicle/Formulation	Solution in 0.9% sodium chloride
Method of Administration	Oral and intravenous bolus
Sample	Plasma
Analyte	FTC
Assay	Validated HPLC with UV detection
Dose (mg/kg)	10 oral/ 10 IV
Sex (M/F)/Number of Animals	120 M / dose

PK Parameters	Oral	IV
t_{max} (min)	25.4	-
C_{max} (µM)	9.8	-
$AUC_{0-\infty}$ (µM•h)	16.7	17.4
CL (L/h/kg)	-	2.33
t½β (min)	-	23
V_{ss} (L/kg)	-	0.89

Additional Information: Absolute bioavailability = 96%

FTC = emtricitabine

3.1.2. TEIN/93/0004: Pharmacokinetics of FTC in Mice (100 mg/kg)

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics of 100 mg/kg or Oral and Intravenous 524W91 in Male CD-1 Mice	Absorption, bioavailability	FTC	TEIN/93/0004

Species	Mouse / CD-1
Feeding Condition	Not fasted
Vehicle/Formulation	Solution in 0.9% sodium chloride
Method of Administration	Oral and intravenous bolus
Sample	Plasma
Analyte	FTC
Assay	Validated HPLC with UV detection
Dose (mg/kg)	100 oral/ 100 IV
Sex (M/F)/Number of Animals	120 M / dose

PK Parameters	Oral	IV
t_{max} (min)	24.5	-
C_{max} (µM))	89	-
$AUC_{0-\infty}$ (µM•h)	143	181
CL (L/h/kg)	-	2.23
t½ beta (min)	-	15.5
t½ gamma (min)	-	82
V_{ss} (L/kg)	-	0.94
Additional Information: Absolute bioavailability = 79%		

FTC = emtricitabine

3.1.3. [redacted]00101: Pharmacokinetics of FTC in Mice (600 mg/kg)

Report Title	Study Type	Test Article	Report Number
Pharmacokinetic Study in Male Mice Following Single Oral and Intravenous Administration of L-(-)-2',3'-Dideoxy-5-Fluoro-3'-Thiacytidine	Absorption, bioavailability	FTC	[redacted]00101

Species	Mouse / CD-1
Sex (M/F)/Number of Animals	Oral – 36 (numbered 4-39, 1-3 were bled predose) (male); IV – 36 (numbered 43-78, 40-42 were bled predose) (male)
Feeding Condition	Not fasted
Vehicle/Formulation	Oral – 60 mg/mL suspension with 0.5% hydroxypropylmethyl cellulose; IV – 60 mg/mL in phosphate buffered saline using distilled water, pH 7.2
Method of Administration	Oral and IV bolus
Dose (mg/kg)	600
Sample	Plasma
Analyte	FTC
Assay	Validated HPLC with UV detection

PK Parameters	Oral	IV
t_{max} (h)	0.667	0
C_{max} (µg/mL))	139	1560
$AUC_{0\text{-}last}$ (µg•h/mL)	270	465
$AUC_{0\text{-}\infty}$ (µg•h/mL)	296	473
CL (L/h/kg)	-	1.28
t½ lambda (h)	3.17	4.14
λ_z (h^{-1})	0.219 (apparent terminal rate constant)	0.167 (apparent terminal rate constant)
V_{ss} (L/kg)	-	1.10
MAT (h)	2.04 (calculated as MRT_{po}-MRT_{IV})	-
MRT (h)	2.90 (calculated from AUMC/ $AUC_{0\text{-}\infty}$)	0.860 (calculated from AUMC/ $AUC_{0\text{-}\infty}$)
F	62.7%	-

FTC = emtricitabine; IV = intravenous

3.1.4. TEZZ/93/0019: Pharmacokinetics of FTC in Fasted Cynomolgus Monkeys

Report Title	**Study Type**	**Test Article**	**Report Number**
A Pharmacokinetic Study of 524W91 in Cynomolgus Monkeys Following Oral and Intravenous Administration	Absorption, bioavailability	FTC	TEZZ/93/0019

Species	Monkey / Cynomolgus
Sex (M/F)/Number of Animals	4 M / dose
Feeding Condition	Fasted
Vehicle/Formulation	Solution in 0.9% sodium chloride
Method of Administration	Oral and intravenous (crossover)
Dose (mg/kg)	10 and 80
Sample	Plasma
Analyte	FTC
Assay	Validated HPLC with UV detection

PK Parameters

Oral	**10 mg/kg**	**80 mg/kg**
C_{max} (µM)	14.1 ± 2.00	111 ± 34.0
t_{max} (h)	1.3 ± 0.50	2.30 ± 0.29
$AUC_{0-\infty}$ (µM•h)	37.4 ± 6.5	285 ± 82.6
CL/F (L/h/kg)	1.1 ± 0.19	1.2 ± 0.31
Intravenous	**10 mg/kg**	**80 mg/kg**
$AUC_{0-\infty}$ (µM•h)	59.9 ± 11.4	493 ± 65.4
CL (L/h/kg)	0.70 ± 0.14	0.70 ± 0.08
Vss (L/kg)	0.80 ± 0.02	0.80 ± 0.09
t½β (h)	1.00 ± 0.19	1.02 ± 0.13

Additional Information: Absolute bioavailability 44 to 69%.

3.1.5. [redacted]00301: Pharmacokinetics of FTC in Nonfasted Cynomolgus Monkeys

Report Title	Study Type	Test Article	Report Number
Pharmacokinetic Study in Male cynomolgus Monkeys Following Single Oral and Intravenous Administration of L-(-)-2',3'-Dideoxy-5-Fluoro-3'-Thiacytidine	Absorption, bioavailability	FTC	[redacted]00301

Species	Monkey / Cynomolgus
Sex (M/F)/Number of Animals	4 M / dose
Feeding Condition	Nonfasted
Vehicle/Formulation	Oral: 32.0 mg/mL aqueous solution, Intravenous: 81.8 mg/mL in phosphate buffered saline, pH 7.2
Method of Administration	Oral and intravenous (crossover)
Dose (mg/kg)	80
Sample	Plasma
Analyte	FTC
Assay	Validated HPLC with UV detection

Oral		Intravenous	
Plasma	Mean ± Standard Deviation	**Plasma**	Mean ± Standard Deviation
C_{max} (µg/mL)	39.4 ± 4.87	**C_{max} (µg/mL)**	238 ± 46.0 (end of infusion)
t_{max} (h)	0.884 (median)	**t_{max} (h)**	Not applicable
$AUC_{0\text{-}last}$ (µg•h/mL)	83.0 ± 11.1	**$AUC_{0\text{-}last}$ (µg•h/mL)**	85.5 ± 17.3
$AUC_{0\text{-}\infty}$ (µg•h/mL)	83.6 ± 11.2	**$AUC_{0\text{-}\infty}$ (µg•h/mL)**	86.1 ± 17.3
CL/F (L/h/kg)	Not calculated	**CL/F (L/h/kg)**	0.970 ± 0.158
t½ lambda (h)	0.936 ± 0.0909	**t½ lambda (h)**	0.775 ± 0.0586
λ_z (h^{-1})	0.746 ± 0.0793	**λ_z (h^{-1})**	0.898 ± 0.0640
MAT (h)	0.953 ± 0.154 (calculated as MRT_{po} - MRT_{iv})	**MRT (h)**	0.802 ± 0.0793 (calculated from $AUMC/AUC_{0\text{-}\infty}$)
MRT (h)	1.75 ± 0.156 (calculated from $AUMC/AUC_{0\text{-}\infty}$)	**Vss (L/kg)**	0.769 ± 0.0743
F (%)	97.4 ± 6.98		

3.2. RPV

3.2.1. TMC278-NC104: Caco-2 Permeability of RPV (In Vitro)

Report Title	Study Type	Test Article	Report Number
Determination of the In Vitro Transport Characteristics of TMC278, Evaluation of the Possible Role of P-glycoprotein in TMC278 Transport and Assessment of Possible Inhibition of P-glycoprotein Activity by TMC278: A Study in Caco-2 Monolayers	Absorption	[^{14}C]RPV	TMC278-NC104

Method:
Caco-2 cells were maintained for 21-23 days on cell culture inserts. ^{14}C-TMC278 (3 - 300 µM) was added to the apical or basolateral side of the monolayers and transport was measured for 15, 45 and 90 minutes. ^{14}C-alniditan, ^{3}H-levocabastine and ^{3}H-theophylline at a final concentration of 20 µM were included as control compounds for low, medium and high permeability, respectively. The possible inhibition of human P-glycoprotein (or other efflux transporters) was assessed after incubation of ^{3}H-taxol (75.8 nM) in absence or presence of TMC278 (1 - 100 µM). Measurement of bidirectional transport in the presence of 100 µM verapamil was used as a positive control.

Condition, Compound	P_{app} (10^{-6} cm/s) ± SD (n=4)	
	Apical to Basolateral	Basolateral to Apical
3 µM TMC278	11.1 ± 2.0	22.8 ± 3.3
10 µM TMC278	12.5 ± 2.2	19.0 ± 3.9
30 µM TMC278	13.4 ± 1.1	26.5 ± 5.7
100 µM TMC278	12.8 ± 1.8	16.1 ± 2.3
300 µM TMC278	9.8 ± 0.8	15.1 ± 3.7
30 µM TMC278 + 100 µM verapamil	11.8 ± 1.6	27.1 ± 4.8
20 µM alniditan	0.6 ± 0.3	0.7 ± 0.0
20 µM levocabastine	20.2 ± 4.2	24.8 ± 0.9
20 µM theophylline	28.4 ± 2.4	31.4 ± 5.6

Additional Information
Apical to Basolateral (absorptive) and Basolateral to Apical (secretory) P_{app} values were calculated from the slopes of 15 - 45 - 90 min transport-time profiles. All incubations in this experiment were conducted in the presence of Hank's Balanced Salt Solution + 10% Fetal Calf Serum, at pH 6.5 (+ 25 mM MES) in the apical compartment and at pH 7.4 in the basolateral compartment. The average mannitol permeability values were below 0.8 x 10^{-6} cm/s.
TMC278 has P-glycoprotein inhibitory properties with an apparent IC_{50} value of 9.2 µM (3.4 µg/mL).

3.2.2. TMC278-FK4259: In Vivo Micronucleus Test on Bone Marrow Cells of Mice

Report Title			Study Type		Test Article	Report Number
In Vivo Micronucleus Test on Bone Marrow Cells of Mice			Absorption		RPV	TMC278-FK4259 [a]
Species	Mouse (CD-1)					
Feeding Condition	Not fasted					
Vehicle/Formulation	TMC278 base in PEG400/CA (10%)					
Route	Oral (gavage)					
Gender (M/F)/Number of Animals	M/6	F/6	M/6	F/6	M/10	F/10
Dose (mg/kg)	100		400		1600	
Concentration (mg/mL)	5		20		80	
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma	
Analyte	TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters						
C1h (µg/mL)	39	33	59	68	60	58
Estimated AUC (µg.h/mL)	158	130	262	258	307	287
(Time for calculation –h)	(0-6)	(0-6)	(0-6)	(0-6)	(0-6)	(0-6)

Additional Information
The sampling times were 1 and 6 h after dose administration.
a the toxicology study number is TOX5538
CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; PEG400: polyethylene glycol 400

3.2.3. TMC278-FK4195: Pharmacokinetics of RPV in Rats (IV/Oral)

Report Title	Study Type	Test Article	Report Number
TMC278-FK4195: Pharmacokinetics and tissue distribution in male Sprague-Dawley rats after single intravenous administration (slow bolus injection) of a PEG400/sterile water (75/25) solution at 4 mg R278474/kg and absorption and tissue distribution after single oral administration of a solution of PEG400 + citric acid (10%) at 40, 160 and 400 mg R278474/kg and of a solution of PEG400 at 40 mg R278474/kg	Absorption	RPV	TMC278-FK4195

Species	Rats (Sprague Dawley)					
Feeding Condition	-					
Vehicle/Formulation	TMC278 base in PEG400/sterile water (25%)	TMC278 base in PEG400	TMC278 base in PEG400/CA (10%)			
Route	Intravenous (slow bolus injection)	Oral (gavage)	Oral (gavage)			
Gender (M/F)/Number of Animals	M/18	M/15	M/15	M/15	M/15	
Dose (mg/kg)	4	40	40	160	400	
Concentration (mg/mL)	2	4	4	16	40	
Sample (whole blood, plasma, serum, etc.)	plasma	plasma	plasma	plasma	plasma	
Analyte	TMC278	TMC278	TMC278	TMC278	TMC278	
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	
Pharmacokinetic Parameters						
C_{max} (µg/mL)	5.3 [a]	1.3	1.7	3.3	6.6	
t_{max} (h)	NA	1.0	1.0	8.0	8.0	
AUC (µg.h/mL)	3.1	9.8	12	48	64	
(Time for calculation –h)	(0-∞)	(0-∞)	(0-∞)	(0-∞)	(0-∞)	
$t_{1/2}$ (h)	4.4	2.8	4.6	5.7	3.2	
(Time for calculation –h)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	
Bioavailability (Fabs %)	NA	32	39	39	21	
Clearance (L/h/kg)	1.3	NA	NA	NA	NA	
Vd_{ss} (L/kg)	4.1	NA	NA	NA	NA	

Additional Information

The sampling times were 7 min (iv only), 20 min, 1, 3, 8 and 24 hours after dose administration.

Tissue samples from the adrenal gland, brain, liver and muscle were collected as well in this study. Maximum tissue concentrations were observed within 20 to 60 min after administration. Tissue levels declined in parallel with plasma concentrations. Tissue to plasma concentration (AUC_{0-24h}) ratios were 3.4, 2.7, 0.49 and 0.45 for liver, adrenal gland, brain and muscle, respectively.

a C_0: extrapolated value at 0 h.

CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; NA: not applicable; PEG400: polyethylene glycol 400; Vd_{ss}: volume of distribution at steady state

3.2.4. TMC278-NC106: Pharmacokinetics of RPV in Rats (Oral)

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics and relative bioavailability of R278474 in SPF Sprague Dawley rats after single oral administration of R278474 at 40 mg base eq./kg given as an oral PEG400 solution R278474.HCl (R314585) and R278474-fumarate salt (R366650) given as a suspension	Absorption	RPV	TMC278-NC106

Species	Rats (Sprague Dawley)		
Feeding Condition	Not fasted		
Vehicle/Formulation	TMC278 base in PEG400	TMC278.HCl in Tween20/HPMC/water	TMC278 fumarate in Tween20/HPMC/water
Route	Oral (gavage)	Oral (gavage)	Oral (gavage)
Gender (M/F)/Number of Animals	M/6	M/6	M/6
Dose (mg/kg)	40	40	40
Concentration (mg/mL)	4	4	4
Sample (whole blood, plasma, serum, etc.)	plasma	plasma	plasma
Analyte	TMC278	TMC278	TMC278
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters			
C1h (µg/mL)	1.9	0.77	1.7
tmax (h)	1.0	6.0	4.0
AUC (µg.h/mL)	10	7.4	11
(Time for calculation –h)	(0-∞)	(0-∞)	(0-∞)
$t_{1/2}$ (h)	6.0	2.0	3.3
(Time for calculation –h)	(8-24)	(8-24)	(8-24)

Additional Information
The sampling times were 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after dose administration.
HPMC: hydroxypropyl methyl cellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry; PEG400: polyethylene glycol 400

3.2.5. TMC278-FK4278: Single Dose Oral Toxicity Study in the Rat

Report Title		**Study Type**	**Test Article**	**Report Number**
Single Dose Oral Toxicity Study in the Rat		Absorption	RPV	TMC278-FK4278 [a]
Species	Rats (Sprague Dawley)			
Feeding Condition	Fasted			
Vehicle/Formulation	TMC278 base in PEG400/CA (10%)			
Route		Oral (gavage)		
Gender (M/F)/Number of Animals	M/4		F/4	
Dose (mg/kg)		800		
Concentration (mg/mL)		40		
Sample (whole blood, plasma, serum, etc.)		plasma		
Analyte		TMC278		
Assay		LC-MS/MS		
Mean Pharmacokinetic Parameters				
C_{max} (µg/mL)	8.0		18	
t_{max} (h)	8.0		8.0	
AUC (µg.h/mL)	86		233	
(Time for calculation –h)	(0-∞)		(0-∞)	
$t_{1/2}$ (h)	5.1		6.3	
(Time for calculation –h)	(8-24)		(8-24)	

Additional Information
The sampling times were 0.3, 1, 3, 8 and 24 h after dose administration.
a the toxicology study number is Exp5559
CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; PEG400: polyethylene glycol 400

3.2.6. TMC278–FK4293: Pharmacokinetics of RPV in Rabbits (IV)

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics of R278474 in female white New Zealand rabbits after single intravenous administration of a 75 % PEG400 / 25 % sterile water solution of R278474 at 1.25, 2.9 and 4 mg/kg	Absorption	RPV	TMC278-FK4293

Species	Rabbits (New Zealand white)		
Feeding Condition	Not fasted		
Vehicle/Formulation	TMC278 base in PEG400/sterile water (25%)		
Route	Intravenous (slow bolus injection)		
Gender (M/F)/Number of Animals	F/3	F/1	F/2
Dose (mg/kg)	1.25[a]	2.9[b]	4
Concentration (mg/mL)		2	
Sample (whole blood, plasma, serum, etc.)		plasma	
Analyte		TMC278	
Assay		LC-MS/MS	
Mean Pharmacokinetic Parameters			
C_0 (µg/mL)[c]	8.5	12	28
AUC (µg.h/mL)	44	104	151
(Time for calculation –h)	(0-∞)	(0-∞)	(0-∞)
$t_{1/2}$ (h)	12	21	21
(Time for calculation –h)	(24-48)	(48-72)	(48-72)
Clearance (L/h/kg)	0.030	0.028	0.027
Vd_{ss} (L/kg)	0.32	0.56	0.45

Additional Information:
The sampling times were 0 (predose), 0.13, 0.25, 0.5, 1, 3, 8, 24, 48 and 72 h (2.9 and 4 mg/kg dose levels only) after dose administration.
a Since dosing of the rabbits with PEG400 formulation at 4 mg/kg, in general, went difficult, an extra 3 animals were dosed at a much lower concentration of 1.25 mg/kg; [b] Originally 3 animals should receive 4 mg/kg; because one animal was difficult to handle it only received 2.9 mg/kg; [c] $C_{0:}$ extrapolated concentrations at 0 h.
LC-MS/MS: liquid chromatography with tandem mass spectrometry; PEG400: polyethylene glycol 400; Vd_{ss}: volume of distribution at steady state

3.2.7. TMC278-FK4231: Pharmacokinetics of RPV in Dogs (IV/Oral)

Report Title	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics and Absolute and Relative Bioavailability in Male Beagle Dogs After Single Intravenous Administration of a 75 % PEG400/25 % Sterile Water Solution of R278474 at 1.25 mg/kg and After Single Oral Administration of Different Formulations of R278474 at 5 mg/kg	Absorption	RPV	TMC278-FK4231

Species	Dog (beagle)		
Feeding Condition	-		
Vehicle/Formulation	TMC278 base in PEG400/sterile water (25%)	TMC278 base in PEG400	TMC278 base in PEG400/CA (10%)
Route	Intravenous (slow bolus injection)	Oral (gavage)	Oral (gavage)
Gender (M/F)/Number of Animals	M/2	M/2	M/2
Dose (mg/kg)	1.25	5	5
Sample (whole blood, plasma, serum, etc.)	plasma	plasma	plasma
Analyte	TMC278	TMC278	TMC278
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters			
C_{max} (µg/mL)	NA	0.34	1.2
t_{max} (h)	NA	4.0	3.0
AUC (µg.h/mL)	8.7	11	28
(Time for calculation –h)	(0-∞)	(0-∞)	(0-∞)
$t_{1/2}$ (h)	31	39	18
(Time for calculation –h)	(8 or 32-72)	(32-72)	(8-72 or 48-72)
Bioavailability (%)		31	80
Clearance (L/h/kg)	0.14	-	-
Vd_{ss} (L/kg)	5.2	-	-

Additional Information
The sampling times were 0, 0.13 (IV only), 0.25 (IV only), 0.5, 1, 2, 4, 6, 8, 24, 32, 48 and 72 h after dose administration.
Other formulations were tested (capsules containing PEG400/CA, CA/cromophor RH40, CA/HPC E5/vitamin E TPGS and CA/HP-β-CD/vitamin E TPGS). Bioavailability after administration of these formulations was <61% except for capsule containing CA/HPC E5/vitamin E TPGS.
CA: citric acid; HPC: hydroxypropylcellulose; HP-β-CD: hydroxypropyl-β-cyclodextrine; LC-MS/MS: liquid chromatography with tandem mass spectrometry; NA: not applicable; PEG400: polyethylene glycol 400; TPGS: d-alpha-tocopheryl polyethylene glycol 1000 succinate; Vd_{ss}: volume of distribution at steady state

3.2.8. TMC278-DM02403: Pharmacokinetics of RPV in Monkeys (IV/Oral)

Report Title	Study Type	Test Article	Report Number
Evaluation of the Oral Bioavailability and Pharmacokinetics of R278474 in Male Cynomolgus Monkeys following Single Oral and Intravenous Bolus Administration	Absorption	RPV	TMC278-DM02403

Species	Monkey (cynomolgus)	
Feeding Condition	Not fasted	
Vehicle/Formulation	TMC278 base in PEG400/sterile water (25%)	TMC278 base in PEG400/CA (10%)
Route	Intravenous (bolus)	Oral (gavage)
Gender (M/F)/Number of Animals	M/4	M/4
Dose (mg/kg)	1.25	5
Concentration (mg/mL)	2	5
Sample (whole blood, plasma, serum, etc.)	plasma	plasma
Analyte	TMC278	TMC278
Assay	LC-MS/MS	LC-MS/MS
Mean Pharmacokinetic Parameters		
C_{max} or C_0 of infusion (µg/mL) [a]	0.50	0.10
t_{max} (h)	-	9.3
AUC (µg.h/mL)	1.4	1.3
(Time for calculation –h)	(0-∞)	(0-∞)
$t_{1/2}$ (h)	7.1	8.4
(Time for calculation –h)	NS	NS
Cl (L/h/kg)	0.93	-
Vd_{ss} (L/kg)	4.2	-
Bioavailability (%)	-	24

Additional Information
The sampling times were 0, 0.13, 0.25, 0.5, 1, 3, 7, 24, 48 and 72 h after intravenous dosing, and 0, 0.5, 1, 2, 4, 7, 24, 48 and 72 h after oral dose administration.
The same animals were used for intravenous and oral administrations after a 2-week washout period.
a $C_{0;}$ extrapolated concentrations at 0 h.
CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; NS: not specified; - : not applicable

3.3. TAF

3.3.1. AD-120-2037: Caco-2 Permeability of TAF (In Vitro)

Report Title	Study Type	Test Article	Report Number
Concentration Dependent Permeability of Tenofovir Alafenamide through Caco-2 Cell Monolayers	Absorption (in vitro)	TAF	AD-120-2037

Bidirectional Permeability of TAF Through Caco-2 Cells

Inhibitor	Direction	Target Concentration (μM)	Initial Conc. (μM)	Recovery (%)	P_{app} (10^{-6} cm/s) Average	Efflux Ratio
–	Cell-Free	10	11.3	106	42.9	20.2
	Forward		10.9	42.7	0.34	
	Reverse		10.0	85.3	6.98	
–	Cell-Free	100	104	112	53.2	13.6
	Forward		92.0	40.4	0.63	
	Reverse		98.1	85.2	8.47	
–	Cell-Free	1000	969	109	46.1	6.28
	Forward		933	77.4	1.08	
	Reverse		1038	88.8	5.86	
CsA	Cell-Free	10	9.85	113	43.5	1.00
	Forward		10.4	42.2	1.51	
	Reverse		10.8	70.5	1.34	

Caco-2 = human colonic adenocarcinoma cell line; TAF = tenofovir alafenamide; P_{app} = apparent permeability; CsA = cyclosporine A

3.3.2. AD-120-2014: Pharmacokinetics of TAF in Mice

Report Title	Study Type	Test Article	Report Number
Collection of Samples for Determination of the Pharmacokinetics of GS-7340-02 and GS-7340-03 After a Single Oral Dose to Mice	Absorption	TAF	AD-120-2014

Species	CD-1 mice
Feeding Condition	Not fasted
Vehicle Formulation	0.1% (v/v) tween 20 and 0.1% (w/v) hydroxypropylmethylcellulose (HPMC) K100LV prepared in reverse osmosis water
Method of Administration	Oral gavage
Sample	Plasma
Assay	LC/MS/MS

Test Article	GS-7340-02						GS-7340-03					
Sex (M/F) / N of Animals	M/36						M/36					
Dose (mg/kg)	10		30		100		10		30		100	
Analyte	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV
PK Parameters												
t_{max} (h)	0.08	0.50	NA	0.25	0.08	0.75	NA	0.50	4.00	0.50	0.25	1.50
C_{max} (ng/mL)	5.53	106	NA	440	37.1	1827	NA	85.4	10.3	383	34.7	2152
$t_{½}$ (h)	NA	NA	NA	NA	NA	NA	NA	5.16	NA	10.1	NA	NA
AUC_{0-t} (ng•h/mL)	NA	455	NA	2005	26.0	10643	NA	493	NA	2477	11.3	10866
t_{last} (h)	NA	12.0	NA	24.0	1.50	24.0	NA	12.0	NA	24.0	0.50	24.0
C_{last} (ng/mL)	NA	21.6	NA	35.9	12.8	157	NA	20.5	NA	34.4	24.9	205

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide; TFV = tenofovir

3.3.3. AD-120-2016: Pharmacokinetics of TAF in Mice

Report Title	Study Type	Test Article	Report Number
Collection of Samples for Determination of the Pharmacokinetics of GS-7340-03 After a Single Oral Gavage Dose to Male and Female 001178-W (wild type) Mice	Absorption	TAF	AD-120-2016

Species	Model 001178-W [Wild, CByB6F1-Tg(HRAS)2Jic] mice
Feeding Condition	Not fasted
Vehicle Formulation	0.1:0.1:99.8 (w/w/w) hydroxypropyl methylcellulose K100LV (HPMC)/polysorbate (tween) 20/reverse osmosis water
Method of Administration	Oral gavage
Sample	Plasma
Assay	LC/MS/MS

Test Article	GS-7340-03											
Sex (M/F) / N of Animals	M/44		F/44		M/44		F/44		M/44		F/44	
Dose (mg/kg)	10				30				100			
Analyte	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV
PK Parameters												
t_{max} (h)	NA	0.25	NA	0.50	0.08	0.25	0.50	0.25	0.25	0.50	0.50	0.50
C_{max} (ng/mL)	NA	175	NA	100	8.80	615	117	421	648	1988	280	1733
$t_{½}$ (h)	NA	9.78	NA	8.20	NA	9.51	NA	10.9	NA	8.04	NA	11.0
AUC_{0-t} (ng•h/mL)	NA	735	NA	354	NA	2639	NA	2053	194	10026	104	7131
t_{last} (h)	NA	24.0	NA	12.0	0.25	24.0	NA	24.0	0.50	24.0	0.50	24.0
C_{last} (ng/mL)	NA	13.5	NA	16.5	5.40	31.1	NA	36.3	61.4	99.9	280	113

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide; TFV = tenofovir

3.3.4. AD-120-2015: Pharmacokinetics of TAF in Rats

Report Title	Study Type	Test Article	Report Number
Collection of Samples for Determination of the Pharmacokinetics of GS-7340-02 and GS-7340-03 After a Single Oral Dose to Rats	Absorption	TAF	AD-120-2015

Species	SD rats
Feeding Condition	Not fasted
Vehicle Formulation	0.1% (v/v) tween 20 and 0.1% (w/v) hydroxypropylmethylcellulose (HPMC) K100LV prepared in reverse osmosis water
Method of Administration	Oral gavage
Sample	Plasma
Assay	LC/MS/MS

Test Article	GS-7340-02						GS-7340-03					
Sex (M/F) / N of Animals	M/3						M/3					
Dose (mg/kg)	5		25		100		5		25		100	
Analyte	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV
PK Parameters												
t_{max} (h)	NA	0.67	NA	0.58	NA	0.83	NA	0.58	NA	0.83	NA	0.67
C_{max} (ng/mL)	NA	32.5	NA	199	NA	1240	NA	39.3	NA	364	NA	1670
$t_{½}$(h)	NA	NA	NA	11.2	NA	10.3	NA	NA	NA	7.89	NA	7.85
$AUC_{0\text{-}t}$ (ng•h/mL)	NA	122	NA	1395	NA	7771	NA	88.5	NA	1810	NA	9759
t_{last} (h)	NA	8.00	NA	24.0	NA	24.0	NA	4.67	NA	24.0	NA	24.0
C_{last} (ng/mL)	NA	10.5	NA	25.1	NA	156	NA	12.6	NA	19.4	NA	113

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide; TFV = tenofovir

3.3.5. R990130: Pharmacokinetics of TAF in Rats

Report Title	Study Type	Test Article	Report Number
Tenofovir (GS-1278) Plasma Pharmacokinetics Following a Single Oral Dose of GS-7340-02 in the Male Albino Rat	Absorption	TAF	R990130

Species	Sprague-Dawley rats
Feeding Condition	Fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Oral gavage
Sample	Plasma
Assay	LC/Fluorescence

Test Article	GS-7340-02			
Sex (M/F) / N of Animals	M/4			
Dose (mg/kg)	6.25	25	100	400
Analyte	TFV			
PK Parameters				
t_{max} (h)	0.26	0.50	0.50	0.25
C_{max} (ng/mL)	104	1220	3870	15800
$t_{½}$ (h)	NA	14.5	16.2	17.3
AUC_{0-t} (ng•h/mL)	160	3270	12200	48300
t_{last} (h)	4.00	20.0	20.0	24.0
$MRT_{0-\infty}$ (h)	NA	20.8	18.9	20.6

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide; TFV = tenofovir

3.3.6. R2000065: Pharmacokinetics of TAF in Rats

Report Title	Study Type	Test Article	Report Number
Comparison of Plasma Pharmacokinetics in Rats of Tenofovir Following Oral Administration of GS-7340-02 or Tenofovir DF as Either a suspension in CMC or a Solution in Citric Acid	Absorption	TAF	R2000065

Species	Sprague-Dawley rats
Feeding Condition	Fasted
Dose (mg/kg)	400
Method of Administration	Oral gavage
Sample	Plasma
Assay	LC/MS/MS

Test Article	GS-7340-02		TDF	
Sex (M/F) / N of Animals	M/3			
Vehicle Formulation	CMC suspension	50 mM citric acid	CMC suspension	50 mM citric acid
Analyte	TFV			
PK Parameters				
t_{max} (h)	0.50	0.25	0.25	0.50
C_{max} (ng/mL)	14229	8418	8101	2699
$t_{½}$ (h)	11.3	11.4	7.21	8.31
AUC_{0-t} (ng•h/mL)	36288	33067	15774	11403
$AUC_{0-\infty}$ (ng•h/mL)	36795	33638	15848	11581
t_{last} (h)	55.0	55.0	55.0	48.0

CMC = carboxymethylcellulose; F = female; M = male; TAF = tenofovir alafenamide; TFV = tenofovir

3.3.7. 99-DDM-1278-001-PK: Pharmacokinetics of TAF in Dogs

Report Title	Study Type	Test Article	Report Number
Analysis of Data from SRI Bioavailability Study M059-98 of GS-7340 in Dogs	Absorption, Bioavailability	TAF and its Diastereoisomers Mixture	99-DDM-1278-001-PK

Species	Beagle dogs
Feeding Condition	Fasted
Vehicle Formulation	Sterile Saline
Method of Administration	IV bolus
Assay	LC/Fluorescence

Test Article	GS-7340-2		
Sex (M/F) / N of Animals	M/5		
Dose (mg/kg)	6.20		
Sample	Plasma		PBMC
Analyte	TAF	TFV	TFV
PK Parameters			
t_{max} (h)	0.00*	0.04	7.6
C_{max} (µg/mL)	8.88*	2.09	26.2
$t_{½}$ (h)	0.13	13.7	>24
AUC_{0-t} (µg•h/mL)	1.46	2.01	448
$AUC_{0-\infty}$ (µg•h/mL)	1.52	2.69	NC
V_z (L/kg)	0.82	NA	NA
CL (L/h/kg)	4.48	NA	NA

F = female; IV = intravenous; M = male; NA = not applicable; NC = not calculated; PBMC = peripheral blood mononuclear cells; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-MP = tenofovir monophosphate; TFV-DP = tenofovir diphosphate
* Extrapolated to time zero

Report Title	Study Type	Test Article	Report Number
Analysis of Data from SRI Bioavailability Study M059-98 of GS-7340 in Dogs	Absorption, Bioavailability	TAF and its Diastereoisomers Mixture	99-DDM-1278-001-PK

Species	Beagle dogs
Feeding Condition	Fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Oral gavage
Assay	LC/Fluorescence

Test Article	GS-7340			GS-7340-2								
Sex (M/F) / N of Animals	M/5											
Dose (mg/kg)	18.0			20.0			4.80			5.00		
Sample	Plasma		PBMC	Plasma		PBMC	Plasma		PBMC	Plasma		PBMC
Analyte	TAF	TFV	TFV	TAF	TFV	TFV	TAF	TFV	TFV	TAF	TFV	TFV
PK Parameters												
t_{max} (h)	0.17	1.00	10.0	0.18	0.60	4.40	0.14	0.63	10.0	0.32	1.65	NA
C_{max} (µg/mL)	6.39	0.83	46.0	5.82	0.97	59.7	1.12	0.26	8.30	1.01	0.32	NA
$t_{½}$ (h)	0.32	23.1	NA	0.33	22.0	NA	0.24	20.1	NA	0.19	11.7	NA
AUC_{0-t} (µg•h/mL)	2.79	4.61	706	3.25	5.19	805	0.44	1.26	120	0.48	1.43	NA
$AUC_{0-\infty}$ (µg•h/mL)	2.93	7.89	NC	3.73	8.38	NC	0.50	2.08	NC	0.50	1.78	NA
F (%)[a]	53.4	16.9	NA	76.3	20.1	NA	42.4	20.7	NA	41.2	17.1	NA

F = female; M = male; NC = not calculated; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-DP = tenofovir diphosphate; TFV-MP = tenofovir monophosphate

a Oral bioavailability (F%) for TAF was calculated based on $AUC_{0-\infty}$ value obtained from intravenous administration of GS-7340-2 in dogs (Report #99-DDM-1278-001-PK); Oral bioavailability for tenofovir (TFV) was calculated based on historical $AUC_{0-\infty}$ value (4.30 µg•h/mL) from intravenous administration of TFV in dogs

Report Title	Study Type	Test Article	Report Number
Analysis of Data from SRI Bioavailability Study M059-98 of GS-7340 in Dogs	Absorption, Bioavailability	TAF and its Diastereoisomers Mixture	99-DDM-1278-001-PK

Species	Beagle dogs
Feeding Condition	Fed
Vehicle Formulation	50 mM citric acid
Method of Administration	Oral gavage
Assay	LC/Fluorescence

Test Article	GS-7340-2		
Sex (M/F) / N of Animals	M/5		
Dose (mg/kg)	5.00		
Sample	Plasma		PBMC
Analyte	TAF	TFV	TFV
PK Parameters			
t_{max} (h)	0.30	1.90	NA
C_{max} (µg/mL)	0.24	0.07	NA
$t_{½}$ (h)	0.91	6.14	NA
AUC_{0-t} (µg•h/mL)	0.16	0.43	NA
$AUC_{0-\infty}$ (µg•h/mL)	0.20	0.53	NA
F (%)[a]	16.5	5.08	NA

F = female; M = male; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-MP = tenofovir monophosphate; TFV-DP = tenofovir diphosphate; PBMC = peripheral blood mononuclear cells; NA = not applicable

a Oral bioavailability (F%) for TAF was calculated based on $AUC_{0-\infty}$ value obtained from intravenous administration of GS-7340-2 in dogs (Report #99-DDM-1278-001-PK). Oral bioavailability for tenofovir (TFV) was calculated based on historical $AUC_{0-\infty}$ value (4.30 µg•h/mL) from intravenous administration of TFV in dogs

Report Title	Study Type	Test Article	Report Number
Analysis of Data from SRI Bioavailability Study M059-98of GS-7340 in Dogs	Absorption, Bioavailability	TAF and its Diastereoisomers Mixture	99-DDM-1278-001-PK

Species	Beagle dogs
Feeding Condition	Fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Oral gavage
Assay	LC/Fluorescence

Test Article	GS-7171				GS-7339		
Sex (M/F) / N of Animals	M/5				M/5		
Dose (mg/kg)	16.0				18.0		
Sample	Plasma			PBMC	Plasma		PBMC
Analyte	TAF	GS-7339	TFV	TFV	GS-7339	TFV	TFV
PK Parameters							
t_{max} (h)	0.17	0.23	0.94	8.00	0.21	1.25	5.60
C_{max} (µg/mL)	2.23	5.62	0.91	32.0	4.77	0.94	19.8
$t_{½}$ (h)	0.58	0.57	20.0	NA	0.49	10.4	NA
AUC_{0-t} (µg•h/mL)	1.11	4.63	5.31	707	2.77	5.08	199
$AUC_{0-\infty}$ (µg•h/mL)	1.63	4.89	8.17	NC	3.20	6.14	NC
F (%)[a]	NA	NA	19.7	NA	NA	13.1	NA

F = female; M = male; NA = not applicable; NC = not calculated; PBMC = peripheral blood mononuclear cells; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-DP = tenofovir diphosphate; TFV-MP = tenofovir monophosphate
Note: GS-7171 contains 1:1 mixture of diastereoisomers TAF and GS-7339
a Oral bioavailability for tenofovir (TFV) was calculated based on historical $AUC_{0-\infty}$ value (4.30 µg•h/mL) from intravenous administration of TFV in dogs

3.3.8. AD-120-2034: Pharmacokinetics of TAF in Dogs

Report Title	Study Type	Test Article	Report Number
Plasma and Liver Pharmacokinetics of Tenofovir Alafenamide (TAF) Following Single Oral Administration in Male Beagle Dogs	Absorption	TAF	AD-120-2034

Species	Beagle dogs
Feeding Condition	Fasted
Vehicle Formulation	0.1% (w/w) hydroxypropylmethylcellulose K100LV (HPMC) K100LV, 0.1% polysorbate 20 in water
Method of Administration	Oral gavage[a]
Dose	10 mg/kg
Assay	LC/MS/MS

Test Article	GS-7340-02				
Sex (M/F) / N of Animals	M/6				
Sample	Plasma		Liver		
Analyte	TAF	TFV	TFV	TFV-MP	TFV-DP
PK Parameters					
t_{max} (h)	0.08	1.00	1.00	4.00	4.00
C_{max} (µg/mL)	3.49	0.64	12.7	12.6	56.4
$t_{½}$ (h)	0.24	>24	15.9	>24	22.3
AUC_{0-t} (µg•h/mL)	1.38	2.72	86.8	258	1017

F = female; M = male; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-DP = tenofovir diphosphate; TFV-MP = tenofovir monophosphate
a Animals were pretreated with pentagastrin 20 minutes prior to the dose administration

3.3.9. P2000087: Pharmacokinetics of TAF in Rhesus Monkeys

Report Title	Study Type	Test Article	Report Number
A Single Dose Pharmacokinetic and Oral Bioavailability Study of GS-7340-02 in Rhesus Monkeys	Absorption, bioavailability	TAF	P2000087

Species	Rhesus monkeys
Feeding Condition	Fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Nasogastric gavage
Sample	Plasma
Assay	LC/MS/MS

Test Article	GS-7340-02											
Dose (mg/kg)	0.5				5				50			
Sex (M/F) / N of Animals	M/3		F/3		M/3		F/3		M/3		F/3	
Analyte	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV
PK Parameters												
t_{max} (h)	0.40	1.00	0.33	1.00	0.80	1.33	0.80	1.30	0.40	1.00	0.70	1.00
C_{max} (ng/mL)	3.92	8.50	1.65	6.94	161	169	88.6	152	5388	1576	2897	1076
$t_{½}$ (h)	NC	4.24	0.61	7.18	0.27	10.0	0.19	12.6	0.22	23.9	0.57	14.8
AUC_{0-t} (ng•h/mL)	1.85	34.0	0.60	45.9	131	1151	59.5	924	5038	13119	2583	6749
$AUC_{0-\infty}$ (ng•h/mL)	NC	43.5	2.47	62.0	77.3	1186	82.0	951	5109	13585	2584	6914
MRT (h)	0.43	6.72	0.59	11.0	0.98	11.8	0.88	11.8	0.77	20.0	0.90	15.9

F = female; M = male; TAF = tenofovir alafenamide; TFV = tenofovir

Report Title	Study Type	Test Article	Report Number
A Single Dose Pharmacokinetic and Oral Bioavailability Study of GS-7340-02 in Rhesus Monkeys	Absorption, bioavailability	TAF	P2000087

Species	Rhesus monkeys
Feeding Condition	Fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Nasogastric gavage
Sample	PBMC
Assay	LC/MS/MS

Test Article	GS-7340-02							
	TFV Extract Concentration				Total Converted TFV Extract Concentration[a]			
Dose (mg/kg)	5		50		5		50	
Sex (M/F) / N of Animals	M/3	F/3	M/3	F/3	M/3	F/3	M/3	F/3
2 h	4.03	3.33	167	60.9	5.37	7.2	311	130
24 h	BLQ	2.18	53.3	57.2	4.46	7.57	137	134
96 h	BLQ	BLQ	21.7	18.2	4.03	4.92	66.7	40.6

BLQ = below the limit of quantitation (2 ng/mL); F = female; M = male; PBMC = peripheral blood mononuclear cell; TAF = tenofovir alafenamide; TFV = tenofovir
a Phosphorylated metabolites of TFV were converted to TFV by treatment with acid phosphatase for 30 minutes at 40ºC

3.3.10. P2000031: Pharmacokinetics of TDF in Rhesus Monkeys

Report Title	Study Type	Test Article	Report Number
A Single Dose Oral Bioavailability Study of TDF in Rhesus Monkeys	Absorption, bioavailability	TDF	P2000031

Species	Monkey
Feeding Condition	Fed & Fasted (oral)
Vehicle Formulation	TDF (Citric acid)
Method of Administration	TDF (oral)
Sample	Plasma
Analyte	TFV
Assay	LC/MS/MS

Dose (mg/kg) (TDF)	**5 (Oral)**		**50 (Oral)**		**250 (Oral)**	
Sex (M/F) No. of Animals	6 (3M, 3F) Fed		6 (3M, 3F) Fed		12 (6M, 6F) Fed + Fasted	
PK Parameters (oral) mean (SD)						
C_{max} (µg/mL)	0.113	(0.042)	1.15	(0.676)	1.68	(1.05)
t_{max} (h)	0.83	(0.408)	1.00	(0.548)	1.08	(0.56)
$AUC_{0-\infty}$ (µg•h/mL)	0.725	(0.125)	6.38	(1.74)	14.8	(7.81)
AUC % Extrapolated	2.82	(0.768)	2.26	(1.00)	2.19	(0.710)
$t½\lambda_z$ (h)	8.23	(1.06)	8.54	(1.14)	8.41	(1.20)
CL/F (mL/h/kg)	3202	(566)	3807	(1191)	6569	(2996)
C_{last} (µg/mL)	0.00169	(0.00027)	0.0118	(0.00615)	0.0366	(0.0102)
t_{last} (h)	44.0	(6.20)	48.0	(0.0)	48.0	(0.0)
V_z/F (mL/kg)	38000	(8538)	47250	(18111)	82600	(50113)
F (%)	32.4	(7.90)	23.7	(7.82)	17.0	(5.66)

F = female; IV = intravenous; M = male; TDF = tenofovir disoproxil fumarate; TFV = tenofovir; SD = standard deviation

Report Title	Study Type	Test Article	Report Number
A Single Dose Oral Bioavailability Study of TDF in Rhesus Monkeys	Absorption, bioavailability	TDF	P2000031

Species	Monkey
Feeding Condition	Fed
Vehicle Formulation	TFV (physiological buffered solution)
Method of Administration	TFV (IV)
Sample	Plasma
Analyte	TFV
Assay	LC/MS/MS

Dose (mg/kg) (TFV)	**5 (IV)**		**30 (IV)**	
Sex (M/F) No. of Animals	6 (3M/3F)		6 (3M/3F)	
Tabulated PK for IV Dose Results mean (SD)				
C_{max} (µg/mL)	13.8	(3.08)	79.0	(12.6)
$AUC_{0-\infty}$ (µg•h/mL)	5.12	(1.15)	38.4	(16.2)
AUC % Extrapolated	0.520	(0.394)	0.199	(0.0841)
$t_{½}\lambda_z$ (h)	5.37	(1.35)	8.79	(2.79)
CL (mL/h/kg)	1031	(301)	888	(315)
C_{last} (µg/mL)	0.00419	(0.00455)	0.00620	(0.00317)
t_{last} (h)	24.0	(7.59)	38.0	(11.8)
V_{ss} (mL/kg)	1188	(312)	930	(146)

Additional information: No statistically significant differences were observed between male and female animals in any treatment group and none were observed between fed and fasted states in the 250 mg/kg TDF dose group. Oral bioavailabilities for the 5, 50 & 250 mg/kg groups were 32.4, 23.7 & 17.0%, respectively.

F = female; M = male; IV = intravenous; SD = standard deviation; TDF = tenofovir disoproxil fumarate; TFV = tenofovir

4. PHARMACOKINETICS: ABSORPTION AFTER REPEATED DOSES

4.1. FTC

4.1.1. TOX-109: Oncogenicity study of FTC in Mice – Toxicokinetics

Report Title	Study Type	Test Article	Report Number
Two-year Oral Oncogenicity Study in CD-1 Mice	Absorption	FTC	TOX-109

Species	Mouse
Feeding Condition	Fed
Vehicle Formulation	0.5% aqueous methylcellulose
Method of Administration	Oral gavage
Sample	Plasma
Analyte	FTC
Assay	LC/MS/MS
Dose (mg/kg/day)	0, 80, 250, 750
Sex (M/F) No. of Animals	3/sex/group on Week 2 & Week 26

Parameters	Week		Daily Dose		
			80 mg/kg	250 mg/kg	750 mg/kg
AUC_{0-24} (µg•h/mL)	**Week 2**	Female	27.59	64.97	209.16
		Male	25.52	91.05	234.58
		Mean	26.56	78.01	221.87
	Week 26	Female	23.74	91.71	322.65
		Male	27.48	90.94	287.3
		Mean	25.61	91.33	304.98
C_{max} (µg/mL)	**Week 2**	Female	10.446	33.373	109.690
		Male	16.200	46.609	88.693
		Mean	13.323	39.991	99.192
	Week 26	Female	20.487	49.058	176.950
		Male	16.265	57.682	143.229
		Mean	18.372	53.370	160.090

4.1.2. TOX-108: Oncogenicity Study of FTC in Rats – Toxicokinetics

Report Title	Study Type	Test Article	Report Number
Two-year Oral Oncogenicity Study of FTC in the Rat	Absorption	FTC	TOX-108

Species	Rat
Feeding Condition	Fed
Vehicle Formulation	0.5% aqueous methylcellulose
Method of Administration	Oral gavage
Sample	Plasma
Analyte	FTC
Assay	LC/MS/MS
Dose (mg/kg/day)	0, 60, 200, 600
Sex (M/F) No. of Animals	3/sex/group on Week 2 & Week 26

Parameters	Week		Daily Dose		
			60 mg/kg	200 mg/kg	600 mg/kg
AUC_{0-24} (µg•h/mL)	**Week 2**	Female	30.91	155.99	260.02
		Male	29.91	97.26	279.68
		Mean	30.41	126.62	269.85
	Week 26	Female	52.53	170.68	404.07
		Male	42.87	137.42	326.77
		Mean	47.70	154.05	365.42
C_{max} (µg/mL)	**Week 2**	Female	11.044	30.991	63.813
		Male	12.380	27.565	59.610
		Mean	11.712	29.278	61.712
	Week 26	Female	15.569	52.168	88.993
		Male	13.996	32.339	73.053
		Mean	14.783	42.254	81.023

4.2. RPV

4.2.1. TMC278-NC118: Two-Week Repeated Dose Toxicity Study in the Swiss Mouse - Toxicokinetics

Report Title			Study Type		Test Article		Report Number
Two-Week Repeated Dose Oral Toxicity Study in the Swiss Mouse			Absorption		RPV		TMC278-NC118
Species	Mouse (CD-1)						
Feeding Condition	-				Not fasted		
Vehicle/Formulation	TMC278.HCl in dietary admixture				TMC278.HCl in HPMC (0.5%)		
Route	Oral (diet)				Oral (gavage)		
Gender (M/F)/Number of Animals	M/6	F/6	M/6	F/6	M/9	F/9	
Dose (mg/kg/day)	40		400		400		
Concentration (mg base eq./mL)	-		-		40		
Duration of Dosing (day)	14		14		14		
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		
Analyte	TMC278		TMC278		TMC278		
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		
Pharmacokinetic Parameters							
C_{max} (µg/mL)	8.9	8.3	57	63	63	94	
t_{max} (hh:min [a] or h [b])	2:00	2:00	6:00	14:00	8.0	2.0	
AUC (µg.h/mL)	150	148	1066	1320	687	1233	
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	
$t_{1/2}$ (h)	NA	NA	NA	NA	NC	NC	
(Time for calculation –h)	-	-	-	-	-	-	

Additional Information
After diet administration, the sampling times were 6:00, 14:00, 18:00 and 2:00. After gavage administration, the sampling times were 1, 2, 4, 6, 8 and 24 hours after dosing.
a o'clock time; b ; after gavage
HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry; NA: not applicable; NC: not calculated

4.2.2. TMC278–NC121: Four-Week Toxicity Study in CB6F1-NonTgrasH2 Mice - Toxicokinetics

Report Title								Study Type	Test Article	Report Number
Four-Week Toxicity Study by Oral Route (Gavage) in CB6F1-NonTgrasH2 Mice								Absorption	RPV	TMC278–NC121
Species	Mouse (CD-1)									
Feeding Condition	Not fasted									
Vehicle/Formulation	TMC278.HCl in HPMC (0.5% w/v)									
Route	Oral (gavage)									
Gender (M/F)/Number of Animals	M/15		F/15		M/15		F/15		M/15	F/15
Dose (mg base eq./kg/day)	20				80				320	
Concentration (mg base eq./mL)	2				8				32	
Duration of Dosing (day)	1	29	1	29	1	29	1	29	1	29	1	29
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		plasma		plasma	plasma
Analyte	TMC278		TMC278		TMC278		TMC278		TMC278	TMC278
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters												
C_{max} (µg/mL)	15	14	14	15	30	30	28	37	45	66	42	69
t_{max} (hh:min [a] or h [b])	1.0	2.0	1.0	1.0	1.0	2.0	1.0	1.0	6.0	6.0	1.0	1.0
AUC (µg.h/mL)	52	64	45	54	199	250	192	240	643	1090	667	942
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	1.9	3.1	1.7	5.1	1.3	1.4	1.5	2.1	4.2	28	4.0	NC
(Time for calculation –h)	(6-12)	(12-24)	(6-12)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	-

Additional Information
The sampling times were 1, 2, 6, 12 and 24 h after dosing
LC-MS/MS: liquid chromatography with tandem mass spectrometry; NC: not calculated; HPMC: hydroxypropyl methylcellulose

4.2.3. TMC278–NC119: Three-Month Repeated Dose Oral Toxicity Study in the Swiss Mouse - Toxicokinetics

Report Title						Study Type		Test Article		Report Number			
Three-Month Repeated Dose Oral Toxicity Study in the Swiss Mouse						Absorption		RPV		TMC278–NC119			
Species	Mouse (CD-1)												
Feeding Condition	Not fasted												
Vehicle/Formulation	TMC278.HCl in HPMC (0.5% w/v)												
Route	Oral (gavage)												
Gender (M/F)/Number of Animals	M/15			F/15			M/15			F/15			M/15			F/15		
Dose (mg base eq./kg/day)				20									80					
Concentration (mg base eq./mL)				2									8					
Duration of Dosing (day)	1	31	87	1	31	87	1	31	87	1	31	87	1	31	87	1	31	87
Sample (whole blood, plasma, serum, etc.)	plasma			plasma			plasma			plasma			plasma			plasma		
Analyte	TMC278			TMC278			TMC278			TMC278			TMC278			TMC278		
Assay	LC-MS/MS			LC-MS/MS			LC-MS/MS			LC-MS/MS			LC-MS/MS			LC-MS/MS		
Pharmacokinetic Parameters																		
C_{max} (µg/mL)	14	14	18	13	18	19	28	38	34	32	37	42						
t_{max} (h)	1.0	1.0	1.0	1.0	1.0	0.5	2.0	1.0	0.5	1.0	2.0	1.0						
AUC (µg.h/mL)	71	61	80	59	74	61	236	263	210	250	313	313						
(Time for calculation –h)	(0-∞)	(0-8)	(0-24)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)						
$t_{1/2}$ (h)	3.0	4.5	1.6	1.9	1.8	2.0	1.6	1.9	2.2	1.6	1.7	1.9						
(Time for calculation –h)	(2-8)	(2-8)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)						

Additional Information
The sampling times were 0.5, 1, 2, 8 and 24 h after dosing.
LC-MS/MS: liquid chromatography with tandem mass spectrometry; HPMC : hydroxypropyl methylcellulose

Report Title					Study Type	Test Article	Report Number
Three-Month Repeated Dose Oral Toxicity Study in the Swiss Mouse					Absorption	RPV	TMC278–NC119
Species	Mouse (CD-1)						
Feeding Condition	Not fasted						
Vehicle/Formulation	TMC278.HCl in HPMC (0.5% w/v)						
Route	Oral (gavage)						
Gender (M/F)/Number of Animals		M/15			F/15		
Dose (mg base eq./kg/day)			320				
Concentration (mg base eq./mL)			32				
Duration of Dosing (day)	1	31	87	1	31	87	
Sample (whole blood, plasma, serum, etc.)		plasma			plasma		
Analyte		TMC278			TMC278		
Assay		LC-MS/MS			LC-MS/MS		
Pharmacokinetic Parameters							
C_{max} (µg/mL)	63	63	61	55	84	90	
t_{max} (h)	8.0	2.0	1.0	8.0	1.0	1.0	
AUC (µg.h/mL)	1010	860	665	707	1170	1360	
(Time for calculation –h)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)	
$t_{1/2}$ (h)	6.8	4.9	2.9	4.1	5.8	7.5	
(Time for calculation –h)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	

Additional Information
The sampling times were 0.5, 1, 2, 8 and 24 h after dosing.
LC-MS/MS: liquid chromatography with tandem mass spectrometry; HPMC : hydroxypropyl methylcellulose

4.2.4. TMC278–NC120: Carcinogenicity Study by Oral Gavage Administration to CD-1 mice for 104 Weeks

Report Title	Study Type	Test Article	Report Number
Carcinogenicity Study by Oral Gavage Administration to CD-1 mice for 104 Weeks	Absorption	RPV	TMC278–NC120

Species	Mouse (CD-1)											
Feeding Condition	Not fasted											
Vehicle/Formulation	TMC278.HCl in HPMC (0.5% w/v)											
Gender (M/F)/Number of Animals	M/15	M/9	F/15	F/9	M/15	M/9	F/15	F/9	M/15	M/9	F/15	F/9
Dose (mg base eq./kg/day)	20				60				160			
Concentration (mg base eq./mL)	2								8			
Duration of Dosing	Day 1	Week 28	Day 1	Week 28	Day 1	Week 28	Day 1	Week 28	Day 1	Week 28	Day 1	Week 28
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		plasma		plasma		plasma	
Analyte	TMC278		TMC278		TMC278		TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters												
C_{max} (µg/mL)	9.9	9.8	13	9.9	23	22	24	29	41	36	38	58
t_{max} (h)	2.0	1.0	1.0	1.0	2.0	1.0	2.0	1.0	6.0	2.0	2.0	1.0
AUC (µg.h/mL)	60	76	61	51	239	230	182	278	440	505	345	766
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	2.0	2.2	2.1	2.7	1.5	2.8	1.7	2.5	1.7	4.6	1.5	3.7
(Time for calculation –h)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)

Additional Information
The sampling times were 1, 2, 6, 12 and 24 h after dosing
In the vehicle group, TMC278 plasma concentrations above the lower limit of quantification (0.002 µg/mL) were found in 10 out of 42 samples (concentrations ranged between 0.002 and 0.012 µg/mL)
LC-MS/MS: liquid chromatography with tandem mass spectrometry; HPMC : hydroxypropyl methylcellulose

4.2.5. TMC278-FK4103: Five-Day Repeated Dose Oral Toxicity Study in the Rat (Investigative Tolerance Study)

Report Title			Study Type	Test Article	Report Number
Five-Day Repeated Dose Oral Toxicity Study in the Rat (Investigative Tolerance Study)			Absorption	RPV	TMC278-FK4103 [a]
Species	Rat (Sprague Dawley)				
Feeding Condition	NS				
Vehicle/Formulation	TMC278 base in PEG400				
Route	Oral (gavage)				
Gender (M/F)/Number of Animals	M/5		M/5		
Dose (mg/kg/day)	40		400		
Concentration (mg/mL)	5		50		
Duration of Dosing (day)	1	5	1	5	
Sample (whole blood, plasma, serum, etc.)	plasma	plasma	plasma	plasma	
Analyte	TMC278	TMC278	TMC278	TMC278	
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	
Mean Pharmacokinetic Parameters					
C_{max} (µg/mL)	1.4		1.8		
t_{max} (h)	0.6		8		
C_{1h} (µg/mL)	1.0	1.8	1.5	2.8	
C_{24h} (µg/mL)	0.039	0.15	0.18	0.56	
AUC (µg.h/mL)	14		28		
(Time for calculation –h)	(0-∞)		(0-∞)		
$t_{1/2}$ (h)	3.6		5.7		
(Time for calculation –h)	(8-24)		(8-24)		

Additional Information
The sampling times on Day 1 were 0.5, 1, 4, 8 and 24h after gavage administration; on Day 5 samples were taken 1 and 24 h after administration.
a the toxicology study number is EXP5463
LC-MS/MS: liquid chromatography with tandem mass spectrometry; NS: not specified; PEG400: polyethylene glycol 400

4.2.6. TMC278-FK4243: Two-Week Repeated Dose Oral Toxicity Study in the Rat - Toxicokinetics

Report Title					**Study Type**		**Test Article**		**Report Number**			
Two-Week Repeated Dose Oral Toxicity Study in the Rat					Absorption		RPV		TMC278-FK4243 [a]			
Species	Rat (Sprague Dawley)											
Feeding Condition	Fasted											
Vehicle/Formulation	TMC278 base in PEG400/CA (10%)											
Route	Oral (gavage)											
Gender (M/F)/Number of Animals	M/4		F/4		M/4		F/4		M/4		F/4	
Dose (mg/kg/day)	40		40		120		120		400		400	
Concentration (mg/mL)	4		4		12		12		40		40	
Duration of Dosing (day)	1	14	1	14	1	14	1	14	1	14	1	14
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		plasma		plasma		plasma	
Analyte	TMC278		TMC278		TMC278		TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters												
C_{max} (µg/mL)	1.6	2.2	3.2	5.7	2.8	3.6	4.6	7.8	10	8.4	14	15
t_{max} (h)	2.0	1.0	1.5	2.5	3.3	2.0	4.5	4.5	8.0	8.0	8.0	8.0
AUC (µg.h/mL)	13	16	29	42	30	35	55	88	86	84	128	152
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	2.7	3.7	3.3	3.8	3.9	3.1	5.0	5.5	2.7	3.2	2.9	3.6
(Time for calculation –h)	(8-24)	(3 or 8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)

Additional Information
The sampling times were 0.3, 1, 3, 8 and 24 h after gavage administration.
a the toxicology study number is TOX5535
CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; PEG400: polyethylene glycol 400

4.2.7. TMC278-NC136: Two-Week Repeated Dose Oral Toxicity Study in the Rat - Toxicokinetics

Report Title				**Study Type**		**Test Article**	**Report Number**	
Two-Week Repeated Dose Oral Toxicity Study in the Rat				Absorption		RPV	TMC278-NC136	
Species	Rat (Sprague Dawley)							
Feeding Condition	Not fasted		Not fasted		Not fasted			
Vehicle/Formulation	TMC278 base in dietary admixture		TMC278.HCl in dietary admixture		TMC278.HCl in HPMC (0.5%)			
Route	Oral (diet)		Oral (diet)		Oral (gavage)			
Gender (M/F)/Number of Animals	M/6	F/6	M/6	F/6	M/6	F/6	M/6	F/6
Dose (mg base eq./kg/day)	400		400		1200		400	
Concentration (mg base eq./mL)	-		-		-		40	
Duration of Dosing (day)	12		12		12		12	
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		plasma	
Analyte	TMC278		TMC278		TMC278		TMC278	
Assay	NS		NS		NS		NS	
Pharmacokinetic Parameters								
C_{max} (µg/mL)	3.7	4.3	5.2	7.2	11	13	7.3	12
t_{max} (hh:min (diet) or h (gavage))	22:00	2:00	22:00	2:00	2:00	22:00	4	2
AUC (µg.h/mL)	57	86	82	137	180	266	51	103
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	NA	NA	NA	NA	NA	NA	3.3	2.0
(Time for calculation –h)	-	-	-	-	-	-	(16-24)	(16-24)

Additional Information
The sampling times were 6:00, 10:00, 14:00, 18:00, 22:00 and 2:00 after diet administration and 0, 1, 2, 4, 8, 12, 16, 20 and 24 h after gavage administration.
LC-MS/MS: liquid chromatography with tandem mass spectrometry; HPMC: hydroxypropyl methylcellulose; NA: not applicable; NS: not specified.

4.2.8. TMC278-NC177: Two-Week Repeated Dose Oral Toxicity Study of R314585 in the Rat - Toxicokinetics

Report Title					**Study Type**	**Test Article**	**Report Number**	
Two-Week Repeated Dose Oral Toxicity Study of R314585 in the Rat					Absorption	RPV	TMC278-NC177	
Species	Rat (Sprague Dawley)							
Feeding Condition	Not fasted							
Vehicle/Formulation	TMC278.HCl in HPMC (0.5%)							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/3		F/3		M/3		F/3	
Dose (mg base eq./kg/day)	400		400		1500		1500	
Concentration (mg base eq./mL)	40		40		150		150	
Duration of Dosing (day)	1	14	1	14	1	14	1	14
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		plasma	
Analyte	TMC278		TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters								
C_{max} (µg/mL)	5.4	5.2	12	12	18	9.5	12	12
t_{max} (h)	5.7	3.3	3.3	1.0	3.3	5.7	3.3	3.3
AUC (µg.h/mL)	50	42	78	96	153	86	152[a]	115
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	2.1	3.1	3.3	3.7	5.5	2.5	2.9[a]	2.1[a]
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS

Additional Information
The sampling times were 1, 8, 12 and 24 h after dosing on Days 1 and 14
a n=2; AUC_{0-24h} (n=3) = 123 µg.h/mL
HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry; NS: not specified

Report Title			Study Type	Test Article	Report Number
Two-Week Repeated Dose Oral Toxicity Study of R314585 in the Rat			Absorption	RPV	TMC278-NC177
Species	Rat (Sprague Dawley)				
Feeding Condition	Not fasted				
Vehicle/Formulation	TMC278.HCl in HPMC (0.5%)				
Route	Oral (gavage)				
Gender (M/F)/Number of Animals		M/3		F/3	
Dose (mg base eq./kg/day)		2000		2000	
Concentration (mg base eq./mL)		200		200	
Duration of Dosing (day)	1	14	1	14	
Sample (whole blood, plasma, serum, etc.)	plasma	plasma	plasma	plasma	
Analyte	TMC278	TMC278	TMC278	TMC278	
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	
Pharmacokinetic Parameters					
C_{max} (µg/mL)	10	7.7	17	14	
t_{max} (h)	8.0	3.3	8.0	3.3	
AUC (µg.h/mL)	103	77	206[a]	147	
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	
$t_{1/2}$ (h)	2.0	2.1	6.7	2.9	
(Time for calculation –h)	NS	NS	NS	NS	

Additional Information
The sampling times were 1, 8, 12 and 24 h after dosing on Days 1 and 14
a n=2; AUC_{0-24h} (n=3) = 208 µg.h/mL
HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry; NS: not specified

4.2.9. TMC278-TOX5692: Four-Week Oral (Gavage) Immunotoxicity Study in the Rat - Toxicokinetics

Report Title					Study Type		Test Article		Report Number	
Four-Week Oral (Gavage) Immunotoxicity Study in the Rat					Absorption		RPV		TMC278-TOX5692	
Species	Rat (Sprague Dawley)									
Feeding Condition	Not fasted									
Vehicle/Formulation	TMC278 base in PEG400/CA (10%)									
Route	Oral (gavage)									
Gender (M/F)/Number of Animals	M/4		F/4		M/4		F/4		M/4	
Dose (mg/kg/day)	10		10		40		40		160	
Concentration (mg/mL)	1		1		4		4		16	
Duration of Dosing (day)	1	29	1	29	1	29	1	29	1	29
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		plasma		plasma	
Analyte	TMC278		TMC278		TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	

Pharmacokinetic Parameters												
C_{max} (µg/mL)	0.94	0.88	2.0	1.6	2.0	2.6	3.2	5.8	6.5	6.7	9.6	8.8
t_{max} (h)	4.0	2.0	1.0	2.0	2.0	8.0	1.0	4.0	8.0	8.0	8.0	8.0
AUC (µg.h/mL)	7.5	7.2	14	14	23	27	30	42	52	51	77	89
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	1.9	2.3	2.1	3.2	3.8	2.7	4.1	3.1	2.5	2.5	2.3	2.7
(Time for calculation –h)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)

Additional Information
The sampling times were 0.5, 1, 2, 4, 8 and 24 h after dosing on Days 1 and 29
CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; PEG400: polyethylene glycol 400

4.2.10. TMC278-NC117: One-Month Repeated Dose Oral Toxicity Study in the Rat - Toxicokinetics

Report Title				Study Type	Test Article	Report Number	
One-Month Repeated Dose Oral Toxicity Study in the Rat				Absorption	RPV	TMC278-NC117	
Species	Rat (Sprague Dawley)						
Feeding Condition	Not fasted						
Vehicle/Formulation	TMC278 base in PEG400/CA (10%)						
Route	Oral (gavage)						
Gender (M/F)/Number of Animals	M/6		F/6		M/6		F/6	
Dose (mg/day)	10		10		400		400	
Concentration (mg/mL)	1		1		40		40	
Duration of Dosing (day)	1	24	1	24	1	24	1	24
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		plasma	
Analyte	TMC278		TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters								
C_{max} (µg/mL)	0.87	0.74	1.5	1.4	8.3	7.7	12	13
t_{max} (h)	2.0	2.0	2.0	2.0	8.0	12	8.0	8.0
AUC (µg.h/mL)	6.3	5.4	10	8.6	101	90	123	149
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	2.9	2.8	3.6	3.1	3.02	2.59	2.21	2.50
(Time for calculation –h)	(8-12)	(6-24)	(8-24)	(8-24)	(12-24)	(12-24)	(12-24)	(12-24)

Additional Information
The sampling times were 0.5, 1, 2, 4, 6, 8, 12 and 24 h after dosing on Days 1 and 24
The dose was given in a daily volume of 1 mL/100g in two administrations (0.5 mL/administration and 1 hour separated).
CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; PEG400: polyethylene glycol 400

Report Title				Study Type	Test Article	Report Number	
One-Month Repeated Dose Oral Toxicity Study in the Rat				Absorption	RPV	TMC278-NC117	
Species	Rat (Sprague Dawley)						
Feeding Condition	Not fasted						
Vehicle/Formulation	TMC278.HCl in HPMC (0.5%)						
Route	Oral (gavage)						
Gender (M/F)/Number of Animals	M/6		F/6		M/6		F/6	
Dose (mg base eq./kg/day)	10		10		400		400	
Concentration (mg base eq./mL)	1		1		40		40	
Duration of Dosing (day)	1	24	1	24	1	24	1	24
Sample (whole blood, plasma, serum, etc.)	Plasma		Plasma		Plasma		Plasma	
Analyte	TMC278		TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters								
C_{max} (µg/mL)	0.90	0.76	1.6	1.7	7.0	4.8	9.5	8.9
t_{max} (h)	2.0	2.0	2.0	2.0	4.0	2.0	4.0	2.0
AUC (µg.h/mL)	5.5	4.5	8.6	7.8	52	33	100	86
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	1.8	2.7	2.1	3.4	1.9	2.5	1.7	1.9
(Time for calculation –h)	(8-12)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(12-24)	(12-24)

Additional Information
The sampling times were 0.5, 1, 2, 4, 6, 8, 12 and 24 h after dosing on Days 1 and 24
The dose was given in a daily volume of 1 mL/100g in two administrations (0.5 mL/administration and 1 hour separated).
HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry

4.2.11. TMC278-NC101: Six-Month Repeated Dose Oral Toxicity in the Rat - Toxicokinetics

Report Title				Study Type		Test Article		Report Number	
Six-Month Repeated Dose Oral Toxicity Study with 1-Month Recovery in the Rat				Absorption		RPV		TMC278-NC101	
Species	Rat (Sprague Dawley)								
Feeding Condition	Not fasted								
Vehicle/Formulation	TMC278 base in PEG400/CA (10%)								
Route	Oral (gavage)								
Gender (M/F)/Number of Animals	M/6	M/5	M/3	F/6	F/6	F/5	M/6	M/5	M/3
Dose (mg/kg/day)		40			40			120	
Concentration (mg/mL)		4			4			12	
Duration of Dosing (day)	1	84	175 [a]	1	84	175 [a]	1	84	175 [a]
Sample (whole blood, plasma, serum, etc.)		Plasma			Plasma			Plasma	
Analyte		TMC278			TMC278			TMC278	
Assay		LC-MS/MS			LC-MS/MS			LC-MS/MS	
Pharmacokinetic Parameters									
C_{max} (µg/mL)	2.9	3.3	1.7	6.5	8.2	6.6	6.4	3.4	3.0
t_{max} (h)	1.0	2.0	2.0	1.0	1.0	1.0	8.0	0.5	10
AUC (µg.h/mL)	19	19	12	32	41	50	53	41	35
(Time for calculation –h)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)
$t_{1/2}$ (h)	2.3	3.9	3.9	4.3	5.3	4.3	2.2	6.3	3.9
(Time for calculation –h)	(8-24)	(8-24)	(10-24)	(8-24)	(8-24)	(10-24)	(8-24)	(8-24)	(10-24)

Additional Information
The sampling times were 0.5, 1, 2, 4, 8 and 24 h after dosing on Days 1 and 84 and 0.5, 1, 2, 4, 6, 10 and 24 h on Day 175.
a Total dosing volume of 10 mL/kg was changed after Day 84 and split in two administrations of 5 mL/kg each, with 1.5 h between the two administrations
CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; PEG400: polyethylene glycol 400

Report Title				Study Type		Test Article		Report Number	
Six-Month Repeated Dose Oral Toxicity Study with 1-Month Recovery in the Rat				Absorption		RPV		TMC278-NC101	
Species	Rat (Sprague Dawley)								
Feeding Condition	Not fasted								
Vehicle/Formulation	TMC278 base in PEG400/CA (10%)								
Route	Oral (gavage)								
Gender (M/F)/Number of Animals	F/6	F/5	F/5	M/6	M/5	M/3	F/6	F/6	F/6
Dose (mg/kg)		120			400			400	
Concentration (mg/mL)		12			40			40	
Duration of Dosing (day)	1	84	175 [a]	1	84	175 [a]	1	84	175 [a]
Sample (whole blood, plasma, serum, etc.)		Plasma			Plasma			Plasma	
Analyte		TMC278			TMC278			TMC278	
Assay		LC-MS/MS			LC-MS/MS			LC-MS/MS	
Pharmacokinetic Parameters									
C_{max} (µg/mL)	8.5	11	8.8	9.1	3.9	6.2	17	15	16
t_{max} (h)	0.50	0.50	0.50	8.0	0.50	10	8.0	8.0	10
AUC (µg.h/mL)	83	100	116	92	56	73	160	184	244
(Time for calculation –h)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)
$t_{1/2}$ (h)	2.6	4.9	5.6	3.5	11	4.4	2.5	4.5	7.4
(Time for calculation –h)	(8-24)	(8-24)	(10-24)	(8-24)	(8-24)	(10-24)	(8-24)	(8-24)	(10-24)

Additional Information
The sampling times were 0.5, 1, 2, 4, 8 and 24 h after dosing on Days 1 and 84 and 0.5, 1, 2, 4, 6, 10 and 24 h on Day 175.
a Total dosing volume of 10 mL/kg was changed after Day 84 and split in two administrations of 5 mL/kg each, with 1.5 h between the two administrations
CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; PEG400: polyethylene glycol 400

4.2.12. TMC278–NC123: Carcinogenicity Study in CD Rats for 104 Weeks - Toxicokinetics

Report Title	Study Type	Test Article	Report Number
TMC278-HCI: Carcinogenicity Study by Oral Gavage Administration to CD Rats for 104 Weeks	Absorption	RPV	TMC278–NC123

Species	Rat (Sprague Dawley)												
Feeding Condition	Not fasted												
Vehicle/Formulation	TMC278.HCl in HPMC (0.5%)												
Route	Oral (gavage)												
Gender (M/F)/Number of Animals	M/9			F/9			M/9			F/9			
Dose (mg base eq./kg/day)			40						200				
Concentration (mg base eq.//mL)			4						20				
Duration of Dosing (day)	1	Week 27	Week 39	1	Week 27	Week 39	1	Week 27	Week 39	1	Week 27	Week 39	
Sample (whole blood, plasma, serum, etc.)	plasma			plasma			plasma			plasma			
Analyte	TMC278			TMC278			TMC278			TMC278			
Assay	LC-MS/MS			LC-MS/MS			LC-MS/MS			LC-MS/MS			
Pharmacokinetic Parameters													
C_{max} (µg/mL)	0.94	0.88	2.0	1.6	2.0	2.6	3.2	5.8	6.5	6.7	9.6	8.8	
t_{max} (h)	4.0	2.0	1.0	2.0	2.0	8.0	1.0	4.0	8.0	8.0	8.0	8.0	
AUC (µg.h/mL)	7.5	7.2	14	14	23	27	30	42	52	51	77	89	
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	
$t_{1/2}$ (h)	1.9	2.3	2.1	3.2	3.8	2.7	4.1	3.1	2.5	2.5	2.3	2.7	
(Time for calculation –h)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	

Additional Information
The sampling times were 0.5, 2, 4, 8, 12 and 24 h after dosing.
In the vehicle group, on Day 1, 2 samples out of 18 (at 12 hours, 0.00132 µg/Ml and 0.00488 µg/Ml) were above the limit of quantification (LOQ, 0.001-0.002 µg/Ml), at Week 27, 17 samples (16 samples < 0.006 µg/Ml and 1 = 0.108 µg/Ml) out of 18 were above LOQ and at Week 39, 7 samples (< 0.019 µg/Ml) out of 18 were above the LOQ.
a not calculated since $C_{24h} > C_{12h}$; [b] $AUC_{0-\infty}$ = NC
HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry; NC: not calculated

Report Title	Study Type	Test Article	Report Number
TMC278-HCI: Carcinogenicity Study by Oral Gavage Administration to CD Rats for 104 Weeks	Absorption	RPV	TMC278–NC123

Species	Rat (Sprague Dawley)												
Feeding Condition	Not fasted												
Vehicle/Formulation	TMC278.HCl in HPMC (0.5%)												
Route	Oral (gavage)												
Gender (M/F)/Number of Animals		M/9		F/9	F/9	F/8	M/9	M/8	M/7		F/9		
Dose (mg/kg/day)			500						1500				
Concentration (mg/mL)			50						150				
Duration of Dosing (day)	1	Week 27	Week 39	1	Week 27	Week 39	1	Week 27	Week 39	1	Week 27	Week 39	
Sample (whole blood, plasma, serum, etc.)		plasma			plasma			plasma			plasma		
Analyte		TMC278			TMC278			TMC278			TMC278		
Assay		LC-MS/MS			LC-MS/MS			LC-MS/MS			LC-MS/MS		
Pharmacokinetic Parameters													
C_{max} (µg/mL)	4.5	2.0	1.8	6.5	5.7	8.5	6.1	2.7	2.2	7.0	10	9.4	
t_{max} (h)	4.0	0.50	0.50	2.0	4.0	0.50	8.0	0.50	2.0	2.0	0.50	0.50	
AUC (µg.h/mL)	45	14	14	63	70	46	58	20	18	81	81	84	
(Time for calculation –h)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)	
$t_{1/2}$ (h)	4.5	7.4	8.0	4.1	NC[a]	11	7.8	5.1	NC[a]	5.1	8.0	3.0	
(Time for calculation –h)	(12-24)	(12-24)	(12-24)	(12-24)	-	(12-24)	(12-24)	(12-24)	-	(12-24)	(12-24)	(12-24)	

Additional Information
The sampling times were 0.5, 2, 4, 8, 12 and 24 h after dosing.
In the vehicle group, on Day 1, 2 samples out of 18 (at 12 hours, 0.00132 µg/mL and 0.00488 µg/mL) were above the limit of quantification (LOQ, 0.001-0.002 µg/mL), at Week 27, 17 samples (16 samples < 0.006 µg/mL and 1 = 0.108 µg/mL) out of 18 were above LOQ and at Week 39, 7 samples (< 0.019 µg/mL) out of 18 were above the LOQ.
a not calculated since $C_{24h} > C_{12h}$
HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry; NC: not calculated

4.2.13. TMC278–NC168: Pre- and Post-Natal Developmental Toxicity Dose Range Finding Study in the Rat

Report Title					**Study Type**	**Test Article**	**Report Number**	
Oral (Gavage) Pre- and Post-Natal Developmental Toxicity and Juvenile Toxicity Dose Range Finding Study in the Rat					Absorption	RPV	TMC278–NC168	
Species	Rat (Sprague Dawley)							
Feeding Condition	Not fasted							
Vehicle/Formulation	TMC278.HCl in HPMC (0.5%)							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/8	F/8	M/7	F/8	M/7	F/7	M/8	F/8
Dose (mg base eq./kg/day)	40		120		400		400	
Concentration (mg base eq./mL)	4		12		40		40	
Age of pups (day)	25		25		25		25	
Duration of Dosing (days)	14		14		14		14	
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		plasma	
Analyte	TMC278		TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters								
C_{max} (µg/mL)	2.6	5.8	3.7	3.6	9.1	7.3	6.4	4.9
t_{max} (h)	1.0	1	4	4	4	4	4	1
AUC (µg.h/mL)	12	18	34	28	50	53	43	39
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	2.7	2.3	4.4	2.6	2.8	2.0	4.1	3.2
(Time for calculation –h)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)

Additional Information
Pups in the first 400 mg/kg come from dams dosed with TMC278 HCl at 400 mg/kg. Pups in the second 400 mg/kg group come from dams dosed with vehicle.
The sampling times were predose, 1, 4 and 8 h after dose administration.
On Day 7 of lactation blood samples were taken from pups (4/group/sex/time point) at predose, 2 and 6 h after dose administration to the dams. In pups dosed by lactation AUC_{0-24h} was 0.62 and 0.74 µg.h/mL at 40 mg/kg, 0.94 and 0.91 µg.h/mL at 120 mg/kg and 1.9 and 1.8 µg.h/mL at 400 mg/kg in males and females, respectively.
HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry

4.2.14. TMC278–NC126: Five-Day Repeated Dose Oral Toxicity Study in the Female Rabbit - Toxicokinetics

Report Title		Study Type	Test Article	Report Number
Five-Day Repeated Dose Oral Toxicity Study in the Female Rabbit		Absorption	RPV	TMC278–NC126
Species	Rabbit (New Zealand white)			
Feeding Condition	-			
Vehicle/Formulation	TMC278 base in aqueous HPMC (0.5%)			
Route	Oral (gavage)			
Gender (M/F)/Number of Animals	F/3	F/3	F/3	
Dose (mg/kg/day)	100	300	1000	
Concentration (mg/mL)	10	30	100	
Duration of Dosing (day)	4	4	4	
Sample (whole blood, plasma, serum, etc.)	plasma	plasma	plasma	
Analyte	TMC278	TMC278	TMC278	
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	
Pharmacokinetic Parameters				
C_{max} (µg/mL)	58	124	138	
t_{max} (h)	8.0	13	19	
AUC (µg.h/mL)	1120	2695	2971	
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	
$t_{1/2}$ (h)	ND	ND	ND	
(Time for calculation –h)	-	-	-	

Additional Information
The sampling times were 0 (predose), 0.5, 1, 2, 4, 8 and 24 h after dosing
HPMC: hydroxypropyl-methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: could not be determined

4.2.15. TMC278–FK4102: Single Dose Escalation Oral Toxicity Study Followed by a 5-day Repeated Dose Oral Toxicity Study in the Beagle Dog (Tolerence Study)

Report Title	Study Type	Test Article	Report Number
Single Dose Escalation Oral Toxicity Study Followed by a 5-day Repeated Dose Oral Toxicity Study in the Beagle Dog (Tolerence Study)	Absorption	RPV	TMC278–FK4102 [a]

Species	Dog (beagle)									
Feeding Condition	-									
Vehicle/Formulation	TMC278 base in PEG400		TMC278 base in PEG400		TMC278 base in PEG400/CA (10%)		TMC278 base in PEG400/CA (10%)			
Route	Oral (gavage)		Oral (gavage)		Oral (gavage)		Oral (gavage)			
Gender (M/F)/Number of Animals	M/1	F/1	M/1	F/1	M/1	F/1	M/1		F/1	
Dose (mg/kg/day)	40		80		80		80			
Concentration (mg/mL)	50		100		100		100			
Duration of Dosing (day)	1		1		1		1	5	1	5
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		plasma			
Analyte	TMC278		TMC278		TMC278		TMC278			
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS			
Pharmacokinetic Parameters										
C_{max} (µg/mL)	1.1	0.95	0.88	0.81	2.1	2.2	3.8	13	2.2	8.0
t_{max} (h)	7.0	4.0	7.0	4.0	7.0	7.0	7.0	24	2.0	24
AUC (µg.h/mL)	41	52	37	47	42	49	78	262	43	154
(Time for calculation –h)	(0-∞)	(0-∞)	(0-∞)	(0-∞)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	12	20	16	24	ND	ND	ND	ND	ND	ND
(Time for calculation –h)	(24-72)	(24-144)	(24-120)	(24-120)	-	-	-	-	-	-

Additional Information
The sampling times were 0.5, 1, 2, 4, 7 and 24 h; additionally at 72 and 144 h after dosing at 40 mg/kg and at 120 h after dosing at 80 mg/kg (PEG400).
a the toxicology study number was Exp5461
CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not determined; PEG400: polyethylene glycol.

4.2.16. TMC278–FK4244: Seven-Day Repeated Dose Oral Toxicity Study in the Beagle Dog - Toxicokinetics

Report Title			Study Type	Test Article	Report Number
Seven-Day Repeated Dose Oral Toxicity Study in the Beagle Dog			Absorption	RPV	TMC278–FK4244[a]
Species	Dog (beagle)				
Feeding Condition	Not fasted				
Vehicle/Formulation	TMC278 base in PEG400/CA (10%)				
Route	Oral (gavage)				
Gender (M/F)/Number of Animals	M/3		M/3		M/3	
Dose (mg/kg/day)	20		20		20	
Concentration (mg/mL)	20		20		20	
Duration of Dosing (day)	1	1	1	1	1	1
Sample (whole blood, plasma, serum, etc.)	plasma	plasma	plasma	plasma	plasma	plasma
Analyte	TMC278	TMC278	TMC278	TMC278	TMC278	TMC278
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters						
C_{max} (µg/mL)	1.6	3.7	1.7	7.7	1.5	7.8
t_{max} (h)	3.0	3.0	5.0	11	10	6.0
AUC (µg.h/mL)	18	39	27	159	19	147
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	3.6	7.8	4.1	11	4.2	19
(Time for calculation –h)	(4-8)	(4-8)	(4-8)	(4-8)	(4-8)	(4-8)

Additional Information
The sampling times were 0, 0.5, 1, 2, 4, 8 and 24 h after dosing on Day 1 and Day 7.
a the toxicology study number was Exp5534
CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; PEG400: polyethylene glycol.

4.2.17. TMC278–TOX5650: One-month Repeated Dose Oral Toxicity Study in the Beagle Dog with 1-Month Recovery

Report Title	Study Type	Test Article	Report Number
One-month Repeated Dose Oral Toxicity Study in the Beagle Dog with 1-Month Recovery	Absorption	RPV	TMC278–TOX5650

Species	Dog (beagle)											
Feeding Condition	Not fasted											
Vehicle/Formulation	TMC278 base in PEG400/CA (10%)											
Route	Oral (gavage)											
Gender (M/F)/Number of Animals	M/3		F/3		M/3		F/3		M/3		F/3	
Dose (mg/kg/day)	5		5		10		10		40		40	
Concentration[b] (mg/mL)												
Duration of Dosing (day)	1	28	1	28	1	28	1	28	1	28	1	28
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		plasma		plasma		plasma	
Analyte	TMC278		TMC278		TMC278		TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters												
C_{max} (µg/mL)	0.94	1.5	1.3	2.0	1.3	5.6	1.3	2.5	2.8	12	2.4	9.5
t_{max} (h)	3.0	2.0	3.0	11	6.0	8.0	2.0	5.0	19	5.0	12	8.0
AUC (µg.h/mL)	13[a]	27	19	37	22[a]	103	14[a]	47	51[a]	204	40[a]	160
(Time for calculation –h)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	33	29	11	181	28	33	21	43	21	48	17	50
(Time for calculation –h)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)

Additional Information
The sampling times were 0, 0.5, 1, 2, 4, 8 and 24 h after dosing on Day 1 and Day 28. On Day 30, 31, 35, 44, 49 and 56 at 8:00.
During the 1-month recovery period the mean through levels decreased from Day 30 to Day 56.
The TMC278 levels were measured in the adrenal gland and plasma at autopsy. Tissue to plasma ratios for the adrenal gland were 1.7 and 1.3 at 5 mg/kg, 1.9 and 2.2 at 10 mg/kg and 3.6 and 1.7 at 40 mg/kg in males and females, respectively.
a $AUC_{0-\infty}$ extrapolation > 45%
CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; PEG400: polyethylene glycol.

4.2.18. TMC278–NC116: One-Month Repeated Dose Oral Toxicity Study in the Beagle Dog - Toxicokinetics

Report Title	Study Type	Test Article	Report Number
One-Month Repeated Dose Oral Toxicity Study in the Beagle Dog	Absorption	RPV	TMC278–NC116

Species	Dog (beagle)							
Feeding Condition	Not fasted							
Vehicle/Formulation	TMC278 base in PEG400/CA (10%)							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/3		F/3		M/3		F/3	
Dose (mg/kg/day)	5				40			
Concentration (mg/mL)	5				40			
Duration of Dosing (day)	1	28	1	28	1	28	1	28
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		plasma	
Analyte	TMC278		TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters								
C_{max} (µg/mL)	1.0	1.4	0.66	1.1	1.0	3.8	3.5	2.5
t_{max} (h)	3.0	2.7	7.3	2.3	17	1.8	5.3	2.7
AUC (µg.h/mL)	12	23	8.1	14	17	73	59	43
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	ND	ND	ND	ND	ND	ND	ND	ND
(Time for calculation –h)	-	-	-	-	-	-	-	-

Additional Information
The sampling times were 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 h after dosing on Day 1 and Day 28.
CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not determined; PEG400: polyethylene glycol

Report Title	Study Type	Test Article	Report Number
One-Month Repeated Dose Oral Toxicity Study in the Beagle Dog	Absorption	RPV	TMC278–NC116

Species	Dog (beagle)							
Feeding Condition	Not fasted							
Vehicle/Formulation	TMC278.HCl in HPMC (0.5%)							
Gender (M/F)/Number of Animals	M/3		F/3		M/3		F/3	
Dose (mg base eq./kg/day)	5				40			
Concentration (mg base eq./mL)	5				40			
Duration of Dosing (day)	1	28	1	28	1	28	1	28
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		plasma	
Analyte	TMC278		TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters								
C_{max} (µg/mL)	0.49	0.89	0.53	0.70	1.3	4.1	1.5	5.8
t_{max} (h)	11	2.0	2.7	1.3	9.3	3.0	18	2.0
AUC (µg.h/mL)	6.9	13	6.2	7.5	20	76	22	81
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	ND	ND	ND	ND	ND	ND	ND	ND
(Time for calculation –h)	-	-	-	-	-	-	-	-

Additional Information
The sampling times were 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 h after dosing on Day 1 and Day 28.
HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not determined.

4.2.19. TMC278–NC115: Six-Month Repeated Dose Oral Toxicity Study with 3-Month Interim Kill in the Beagle Dog

Report Title				Study Type	Test Article	Report Number
Six-Month Repeated Dose Oral Toxicity Study with 3-Month Interim Kill in the Beagle Dog				Absorption	RPV	TMC278–NC115
Species	**Dog (beagle)**					
Feeding Condition	**Not fasted**					
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**					
Route	**Oral (gavage)**					
Gender (M/F)/Number of Animals		M/3			F/3	
Dose (mg/kg/day)			5			
Concentration (mg/mL)			5			
Duration of Dosing (day)	1	86	177	1	86	177
Sample (whole blood, plasma, serum, etc.)		plasma			plasma	
Analyte		TMC278			TMC278	
Assay		LC-MS/MS			LC-MS/MS	
Pharmacokinetic Parameters						
C_{max} (µg/mL)	1.0	1.4	1.5	0.70	1.1	1.4
t_{max} (h)	2.0	2.3	1.7	2.3	3.3	1.7
AUC (µg.h/mL)	11	21	21	9.2	18	17
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	ND	ND	ND	ND	ND	ND
(Time for calculation –h)	-	-	-	-	-	-

Additional Information
The sampling times were 0, 1, 2, 4, 8 and 24 h after dosing at Day 1, Day 86 and Day 177.
The TMC278 levels were measured in the liver, adrenal gland and plasma at autopsy at Day 93 and Day 184(males)/185(females). Tissue to plasma ratios for the liver were 8.1/8.9 and 13/13 at 5 mg/kg, 9.7/11 and 9.1/12 at 10 mg/kg and 7.7/9.6 and 9.9/9.4 at 40 mg/kg in males and females on Day93/Day184 or 185, respectively. Tissue to plasma ratios for the adrenal gland were 4.1/5.2 and 5.4/4.1 at 5 mg/kg, 8.1/4.7 and 4.6/4.2 at 10 mg/kg and 5.4/6.8 and 7.4/5.7 at 40 mg/kg in males and females on Day93/Day184 or 185, respectively.
CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not determined; PEG400: polyethylene glycol.

Report Title				Study Type	Test Article	Report Number
Six-Month Repeated Dose Oral Toxicity Study with 3-Month Interim Kill in the Beagle Dog				Absorption	RPV	TMC278–NC115
Species	**Dog (beagle)**					
Feeding Condition	**Not fasted**					
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**					
Route	**Oral (gavage)**					
Gender (M/F)/Number of Animals		M/3			F/3	
Dose (mg/kg/day)			10			
Concentration (mg/mL)			10			
Duration of Dosing (day)	1	86	177	1	86	177
Sample (whole blood, plasma, serum, etc.)		plasma			plasma	
Analyte		TMC278			TMC278	
Assay		LC-MS/MS			LC-MS/MS	
Pharmacokinetic Parameters						
C_{max} (µg/mL)	1.3	2.0	2.0	1.2	2.2	1.9
t_{max} (h)	6.0	2.3	4.0	2.7	2.0	4.0
AUC (µg.h/mL)	22	28	26	20	27	32
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	ND	ND	ND	ND	ND	ND
(Time for calculation –h)	-	-	-	-	-	-

Additional Information
The sampling times were 0, 1, 2, 4, 8 and 24 h after dosing at Day 1, Day 86 and Day 177.
CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not determined; PEG400: polyethylene glycol.

Report Title				Study Type	Test Article	Report Number
Six-Month Repeated Dose Oral Toxicity Study with 3-Month Interim Kill in the Beagle Dog				Absorption	RPV	TMC278–NC115
Species	**Dog (beagle)**					
Feeding Condition	**Not fasted**					
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**					
Route	**Oral (gavage)**					
Gender (M/F)/Number of Animals		M/3			F/3	
Dose (mg/kg/day)			40			
Concentration (mg/mL)			40			
Duration of Dosing (day)	1	86	177	1	86	177
Sample (whole blood, plasma, serum, etc.)		plasma			plasma	
Analyte		TMC278			TMC278	
Assay		LC-MS/MS			LC-MS/MS	
Pharmacokinetic Parameters						
C_{max} (µg/mL)	1.5	2.5	4.0	0.82	3.1	2.9
t_{max} (h)	12	2.0	2.7	9.3	2.7	2.0
AUC (µg.h/mL)	23	41	68	10	52	43
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	ND	ND	ND	ND	ND	ND
(Time for calculation –h)	-	-	-	-	-	-

Additional Information
The sampling times were 0, 1, 2, 4, 8 and 24 h after dosing at Day 1, Day 86 and Day 177.
CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not determined; PEG400: polyethylene glycol.

4.2.20. TMC278–NC107: Fifty-Two Week Oral (Gavage) Toxicity Study in the Beagle Dog

Report Title	Study Type	Test Article	Report Number
Fifty-Two Week Oral (Gavage) Toxicity Study in the Beagle Dog	Absorption	RPV	TMC278–NC107

Species	Dog (beagle)							
Feeding Condition	Not fasted							
Vehicle/Formulation	TMC278 base in PEG400/CA (10%)							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/4				F/4			
Dose (mg/kg/day)	5							
Concentration (mg/mL)	5							
Duration of Dosing (day)	1	90	273	363	1	90	273	363
Sample (whole blood, plasma, serum, etc.)	plasma				plasma			
Analyte	TMC278				TMC278			
Assay	LC-MS/MS				LC-MS/MS			
Pharmacokinetic Parameters								
C_{max} (µg/mL)	0.70	1.2	1.1	1.1	0.75	1.4	1.1	1.5
t_{max} (h)	2.0	0.50	1.0	9.0	2.0	1.1	0.88	1.5
AUC (µg.h/mL)	11	18	16	17	9.7	21	18	19
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	ND	ND	ND	ND	ND	ND	ND	ND
(Time for calculation –h)	-	-	-	-	-	-	-	-

Additional Information
The sampling times were 0, 0.5, 1, 2, 4, 8 and 24 h after dosing
CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not determined; PEG400: polyethylene glycol.

Report Title				Study Type	Test Article	Report Number		
Fifty-Two Week Oral (Gavage) Toxicity Study in the Beagle Dog				Absorption	RPV	TMC278–NC107		
Species	Dog (beagle)							
Feeding Condition	Not fasted							
Vehicle/Formulation	TMC278 base in PEG400/CA (10%)							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/4				F/4			
Dose (mg/kg/day)	10							
Concentration (mg/mL)	10							
Duration of Dosing (day)	1	90	273	363	1	90	273	363
Sample (whole blood, plasma, serum, etc.)	plasma				plasma			
Analyte	TMC278				TMC278			
Assay	LC-MS/MS				LC-MS/MS			
Pharmacokinetic Parameters								
C_{max} (µg/mL)	0.90	2.0	1.3 [b]	1.3 [c]	1.2 [a]	2.3 [b]	2.3 [d]	2.2 [e]
t_{max} (h)	1.5	0.38	1.3 [b]	4.0 [c]	2.8 [a]	0.33 [b]	2.3 [d]	7.0 [e]
AUC (µg.h/mL)	15	29	19 [b]	24 [c]	15 [a]	29 [b]	31 [d]	36 [e]
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	ND	ND	ND	ND	ND	ND	ND	ND
(Time for calculation –h)	-	-	-	-	-	-	-	-

Additional Information
The sampling times were 0, 0.5, 1, 2, 4, 8 and 24 h after dosing
LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not determined; PEG400: polyethylene glycol 400;
a: n=5, one animal (number 133) replaced animal 124 that was killed on Day 20, and received the first dose on Day 28 and was sampled with the other animals at Day 89 (61 days of dosing) ; b: n=3; c: n=2; d: one animal was sampled at day 245;e: one animal was sampled at day 335

Report Title				Study Type	Test Article			Report Number
Fifty-Two Week Oral (Gavage) Toxicity Study in the Beagle Dog				Absorption	RPV			TMC278–NC107
Species	Dog (beagle)							
Feeding Condition	Not fasted							
Vehicle/Formulation	TMC278 base in PEG400/CA (10%)							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/4				F/4			
Dose (mg/kg/day)	40							
Concentration (mg/mL)	40							
Duration of Dosing (day)	1	90	273	363	1	90	273	363
Sample (whole blood, plasma, serum, etc.)	plasma				plasma			
Analyte	TMC278				TMC278			
Assay	LC-MS/MS				LC-MS/MS			
Pharmacokinetic Parameters								
C_{max} (µg/mL)	2.4	3.6	3.2	4.1	2.5	5.5 [a]	3.5 [a]	5.5 [a]
t_{max} (h)	14	1.8	3.0	3.7	8.0	0.67 [a]	4.3 [a]	2.7 [a]
AUC (µg.h/mL)	37	60	60	65	41	88 [a]	51 [a]	61 [a]
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	ND	ND	ND	ND	ND	ND	ND	ND
(Time for calculation –h)	-	-	-	-	-	-	-	-

Additional Information
The sampling times were 0, 0.5, 1, 2, 4, 8 and 24 h after dosing
LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not determined; PEG400: polyethylene glycol 400;
a n=3

4.2.21. TMC278–NC249: Oral (Gavage) Pharmacokinetic Study of TMC278 HCl in the Female Cynomolgus Monkey

Report Title	Study Type	Test Article	Report Number
Oral (Gavage) Pharmacokinetic Study of TMC278 HCl in the Female Cynomolgus Monkey	Absorption	RPV	TMC278-NC249

Species	Monkey (cynomolgus)			
Feeding Condition	Not fasted			
Vehicle/Formulation	TMC278.HCl in HPMC (0.5%)			
Route	Oral (gavage)			
Gender (M/F)/Number of Animals	F/3		F/3	
Dose (mg base eq./kg/day)	10		100	
Concentration (mg base eq./mL)	2		20	
Duration of Dosing (day)	1	7	1	7
Sample (whole blood, plasma, serum, etc.)	plasma	plasma	plasma	plasma
Analyte	TMC278	TMC278	TMC278	TMC278
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters				
C_{max} (µg/mL)	0.086	0.041	0.39	0.48
t_{max} (h)	2.7	3.3	5.3	4.7
AUC (µg.h/mL)	0.88 [a]	0.45	5.1 [b]	5.3
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	7.3	13	121	11
(Time for calculation –h)	(8-24)	(8-24)	(8-24)	(8-24)

Additional Information
The sampling times were 0, 1, 2, 4, 8 and 24 h after dosing
a $AUC_{0-\infty}$ = 0.99 µg.h/mL; [b] $AUC_{0-\infty}$ extrapolation > 25%
HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry

4.2.22. TMC278–NC326: Pharmacokinetics of TMC278 after Single and Repeated Oral Administration of TMC278 HCl in Female Monkeys

Report Title				**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics of TMC278 after Single and Repeated Oral Administration of TMC278 HCl in Female Monkeys				Absorption	RPV	TMC278–NC326
Species	Monkey (cynomolgus)					
Feeding Condition	Not fasted					
Vehicle/Formulation	TMC278.HCl in HPMC (1% w/v) with 0.5% Tween 20					
Route	Oral (gavage)					
Gender (M/F)/Number of Animals	F/3	F/3	F/3		F/3	
Dose (mg base eq./kg/day)	250 b.i.d [a]	500 q.d.	100 b.i.d [a]		250 b.i.d [a]	
Concentration (mg base eq./mL)	50 b.i.d. [a]	50 q.d.	20 b.i.d. [a]		50 b.i.d. [a]	
Duration of Dosing (day)	1	1	1	14	1	14
Sample (whole blood, plasma, serum, etc.)	plasma	plasma	plasma	plasma	plasma	plasma
Analyte	TMC278	TMC278	TMC278	TMC278	TMC278	TMC278
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters						
C_{max1} (µg/mL)	0.33	0.58	0.094	0.29	0.25	0.50
t_{max1} (h)	6.7	6.7	8.0	3.0	5.3	4.0
C_{max2} (µg/mL)	0.67	-	0.19	0.21	0.38	0.56
t_{max2} (h)	5.3	-	5.3	3.7	6.7	4.3
AUC (µg.h/mL)	10.1	6.1	2.8	4.4	5.9	10
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	8.6	8.2	33	19	5.8	9.5
(Time for calculation –h)	(16-24)	(8-24)	(16-24)	(16-24)	(16-24)	(16-24)

Additional Information
The sampling times were 0, 1, 2, 4, 8 (before second dose), 9, 10, 12, 16 and 24 h after b.i.d dosing and 0, 1, 2, 4, 8 and 24 h after q.d. dosing
In the control-dosed group, 3 plasma concentrations, only on day 1, were above the limit of quantification (0.001 µg/mL): at 1 h after dosing (0.00155 and 0.00194 µg/mL) and at 10 h after dosing (0.084 µg/mL)
a twice daily dosing with an interval of 8 hours;
HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry; q.d.: once daily; b.i.d.: twice daily;
C_{max1} and t_{max1}: after the first dose; C_{max2} and t_{max2}: after the second dose

4.2.23. TMC278–NC248: Endocrinological Oral (Gavage) 8-Week Study in the Female Sexually Immature Monkey

Report Title	Study Type	Test Article	Report Number
Endocrinological Oral (Gavage) 8-Week Study in the Female Sexually Immature Cynomolgus Monkey	Absorption	RPV	TMC278–NC248

Species	Monkey (cynomolgus)	
Feeding Condition	Not fasted	
Vehicle/Formulation	TMC278.HCl in HPMC (1% w/v) with 0.5% Tween 20	
Route	Oral (gavage)	
Gender (M/F)/Number of Animals	F/8	F/7
Dose[b] (mg/kg/day)	100 b.i.d.	250 b.i.d.
Concentration (mg/mL)	20 b.i.d.	50 b.i.d.
Duration of Dosing (day)	55	55
Sample (whole blood, plasma, serum, etc.)	plasma	plasma
Analyte	TMC278	TMC278
Assay	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters		
C_{max1} (µg/mL)	0.14	0.31
t_{max1} (h)	2.5	4.6
C_{max2} (µg/mL)	0.18	0.31
t_{max2} (h)	5.5	4.6
AUC (µg.h/mL)	2.7	4.6
(Time for calculation –h)	(0-24)	(0-24)
$t_{1/2}$ (h)	7.8	7.5
(Time for calculation –h)	(16-24)	(16-24)

Additional Information
The sampling times were 0, 1, 2, 4, 8 (before second dose), 9, 10, 12, 16 and 24 h after first dose.
In the control-dosed group, plasma concentrations (26 out of 80) were above the limit of quantification (0.001 µg/mL) and ranged from 0.001 to 0.003 µg/mL
b.i.d.: twice daily; HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry
C_{max1} and t_{max1}: after the first dose; C_{max2} and t_{max2}: after the second dose.

4.3. TAF

4.3.1. AD-120-2033: 7-Day Repeated Dose Study of TAF in Dogs – Pharmacokinetics

Report Title	Study Type	Test Article	Report Number
Plasma and Liver Pharmacokinetics of Tenofovir Alafenamide (GS-7340) Following 7-Day Oral Administration in Male Beagle Dogs	Absorption	TAF	AD-120-2033

Species	Beagle dogs
Feeding Condition	Not fasted
Vehicle Formulation	0.1% (w/w) hydroxypropylmethylcellulose K100LV (HPMC) K100LV, 0.1% polysorbate 20 in water
Method of Administration	Oral Gavage
Dose	8.29 mg/kg/Day
Assay	LC/MS/MS

Test Article	GS-7340-02						
Sex (M/F) / N of Animals	M/4						
Sample	Plasma				Liver		
Sample Collection Time	Day 1		Day 7		Day 7		
Analyte	TAF	TFV	TAF	TFV	TFV	TFV-MP	TFV-DP
PK Parameters							
t_{max} (h)	0.17	0.75	0.17	0.75	4.00	4.00	4.00
C_{max} (µg/mL)	2.15	0.42	1.12	0.61	4.34	27.3	108
$t_{½}$ (h)	0.30	15.7	0.28	19.2	NA	NA	NA
AUC_{0-t} (µg•h/mL)	0.88	1.96	0.37	3.39	NA	NA	NA

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-DP = tenofovir diphosphate; TFV-MP = tenofovir monophosphate

4.3.2. D990175-PK: 28-Day Toxicity Study of TAF in Dogs – Toxicokinetics

Report Title	Study Type	Test Article	Report Number
Toxicokinetics of a 28-Day Oral Gavage Toxicity Study of GS-7340-02 in the Beagle Dog	Absorption	TAF	D990175-PK

Species	Beagle dogs
Feeding Condition	Not fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Oral Gavage
Sample	Plasma
Analyte	TAF
Assay	LC/MS/MS

Test Article	GS-7340-02											
Dose (mg/kg/day)	1.0				3.0				10.0			
Sex (M/F) / N of Animals	M/4		F/4		M/4		F/4		M/4		F/4	
Sample Collection Time	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
PK Parameters												
t_{max} (h)	0.33	0.42	0.25	0.33	0.31	0.31	0.50	0.50	0.44	0.44	0.38	0.38
C_{max} (µg/mL)	0.02	0.03	0.02	0.05	0.05	0.11	0.06	0.10	0.86	2.07	0.75	1.01
$t_{½}$ (h)	NA	NA	NA	NA	NA	NA	NA	NA	0.31	0.65	0.33	0.56
AUC_{0-t} (µg•h/mL)	NA	NA	NA	NA	NA	NA	NA	NA	0.60	NA	0.41	NA
$AUC_{0-\tau}$(µg•h/mL)	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.30	NA	0.86
T_{last} (h)	NA	NA	NA	NA	NA	NA	NA	NA	1.50	3.00	1.00	3.00
C_{last} (ng/mL)	NA	NA	NA	NA	NA	NA	NA	NA	0.08	0.07	0.18	0.05

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide; TFV = tenofovir

Report Title	Study Type	Test Article	Report Number
Toxicokinetics of a 28-Day Oral Gavage Toxicity Study of GS-7340-02 in the Beagle Dog	Absorption	TAF	D990175-PK

Species	Beagle dogs
Feeding Condition	Not fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Oral Gavage
Sample	Plasma
Analyte	TFV
Assay	LC/MS/MS

Test Article	GS-7340-02											
Dose (mg/kg/Day)	1.0				3.0				10.0			
Sex (M/F) / N of Animals	M/4		F/4		M/4		F/4		M/4		F/4	
Sample Collection Time	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
PK Parameters												
t_{max} (h)	NA	3.50	NA	4.75	1.13	1.25	1.75	0.88	1.25	0.81	0.75	0.63
C_{max} (µg/mL)	NA	0.06	NA	0.07	0.12	0.09	0.15	0.13	0.38	0.55	0.40	0.43
$t_{½}$ (h)	NA	NA	NA	NA	NA	NA	NA	NA	16.3	>24	16.6	>24
AUC_{0-t} (µg•h/mL)	NA	NA	NA	NA	NA	NA	NA	NA	1.85	NA	1.78	NA
$AUC_{0-\tau}$(µg•h/mL)	NA	NA	NA	NA	NA	NA	NA	NA	NA	5.45	NA	5.13
T_{last} (h)	NA	NA	NA	NA	NA	NA	NA	NA	21.0	24.0	18.0	24.0
C_{last} (ng/mL)	NA	NA	NA	NA	NA	NA	NA	NA	0.03	0.15	0.05	0.16

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide; TFV = tenofovir

Report Title	Study Type	Test Article	Report Number
Toxicokinetics of a 28-Day Oral Gavage Toxicity Study of GS-7340-02 in the Beagle Dog	Absorption	TAF	D990175-PK

Species	Beagle dogs
Feeding Condition	Not fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Oral Gavage
Sample	PBMC
Analyte	TFV
Assay	LC/MS/MS

Test Article	GS-7340-02					
Dose (mg/kg/Day)	1.0		3.0		10.0	
Sex (M/F) / N of Animals	M/4	F/4	M/4	F/4	M/4	F/4
Intracellular Concentration						
$C_{Day\ 1}$ (µg/mL)	NA	NA	NA	NA	NA	NA
$C_{Day\ 8}$ (µg/mL)	NA	NA	NA	1.80	6.92	7.85
$C_{Day\ 22}$ (µg/mL)	NA	NA	1.70	NA	10.6	10.2
$C_{Day\ 28}$ (µg/mL)	NA	NA	2.44	3.81	19.4	17.5

F = female; M = male; NA = not applicable; PBMC = peripheral blood mononuclear cells; TAF = tenofovir alafenamide; TFV = tenofovir

4.3.3. P2000114-PK: 28-Day Toxicity Study of TAF in Monkeys – Toxicokinetics

Report Title	Study Type	Test Article	Report Number
Toxicokinetics from a 28 Day Toxicity Study of GS-7340-02 and Tenofovir (GS-1278) Administered Orally to Rhesus Monkeys	Absorption	TAF	P2000114-PK

Species	Rhesus monkeys
Feeding Condition	Fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Nasogastric gavage
Sample	Plasma
Assay	LC/MS/MS

Test Article	GS-7340-02															
Dose (mg/kg/Day)	3								30							
Sex (M/F) / N of Animals	M/3				F/3				M/3				F/3			
Sample Collection Time	Day 1		Day 28		Day 1		Day 28		Day 1		Day 28		Day 1		Day 28	
Analyte	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV
PK Parameters																
t_{max} (h)	1.00	1.00	1.00	2.30	1.00	1.00	0.83	1.00	1.00	1.00	0.50	0.75	1.00	1.00	0.50	0.67
C_{max} (µg/mL)	0.003	0.09	0.01	0.05	0.005	0.09	0.02	0.05	0.49	0.96	2.21	1.24	2.29	0.85	0.82	0.78
$t_{½}$ (h)	NA	7.77	NA	12.9	NA	8.41	NA	14.2	NA	14.8	0.18	15.6	NA	10.3	0.43	16.4
$AUC_{0-\infty}$ (µg•h/mL)	NA	0.45	NA	NA	NA	0.49	NA	NA	NA	8.70	NA	NA	NA	4.63	NA	NA
$AUC_{0-\tau}$ (µg•h/mL)	NA	NA	NA	0.32	NA	NA	NA	0.38	NA	NA	1.53	7.36	NA	NA	0.70	3.89
$MRT_{0-\infty}$ (h)	NA	9.38	NA	16.6	NA	10.8	NA	17.4	NA	19.9	0.71	17.7	NA	12.9	2.61	20.2

F = female; M = male; NA = not applicable; PBMC = peripheral blood mononuclear cells; TAF = tenofovir alafenamide; TFV = tenofovir

Report Title	Study Type	Test Article	Report Number
Toxicokinetics from a 28 Day Toxicity Study of GS-7340-02 and Tenofovir (GS-1278) Administered Orally to Rhesus Monkeys	Absorption	TAF	P2000114-PK

Species	Rhesus monkeys
Feeding Condition	Fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Nasogastric gavage
Sample	Plasma
Analyte	TFV
Assay	LC/MS/MS

Test Article	TFV			
Dose (mg/kg/Day)	15			
Sex (M/F) / N of Animals	M/3		F/3	
Sample Collection Time	Day 1	Day 28	Day 1	Day 28
PK Parameters				
t_{max} (h)	1.00	1.5	2.00	2.17
C_{max} (µg/mL)	0.16	0.15	0.12	0.18
$t_{½}$ (h)	6.79	17.6	11.2	12.6
$AUC_{0-\infty}$ (µg•h/mL)	1.01	NA	1.01	NA
$AUC_{0-\tau}$ (µg•h/mL)	NA	1.19	NA	1.46
$MRT_{0-\infty}$ (h)	8.83	21.8	16.0	16.8

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide; TFV = tenofovir

Report Title	Study Type	Test Article	Report Number
Toxicokinetics from a 28 Day Toxicity Study of GS-7340-02 and Tenofovir (GS-1278) Administered Orally to Rhesus Monkeys	Absorption	TAF	P2000114-PK

Species	Beagle dogs
Feeding Condition	Fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Nasogastric gavage
Sample	PBMC
Analyte	TFV
Assay	LC/MS/MS

Test Article	GS-7340-02				TFV	
Dose (mg/kg/Day)	3		30		15	
Sex (M/F) / N of Animals	M/3	F/3	M/3	F/3	M/3	F/3
Intracellular concentrations						
C_{Day14} (µg/mL)	NA	NA	70.9	73.6	NA	NA
C_{Day28} (µg/mL)	NA	NA	31.1	24.6	NA	NA

F = female; M = male; NA = not applicable; PBMC = peripheral blood mononuclear cells; TAF = tenofovir alafenamide; TFV = tenofovir

5. PHARMACOKINETICS: ORGAN DISTRIBUTION STUDIES

5.1. FTC

5.1.1. TOX092: Tissue Distribution and Excretion Study of [^{14}C]FTC in Rats

Report Title	Study Type	Test Article	Report Number
[^{14}C]TP-0006: A Tissue Distribution and Excretion Study in Rats	Tissue Distribution, Excretion	[^{14}C]FTC	TOX092

Species	Rat
Sex (M/F) No. of Animals	20 M Sprague-Dawley group 1, 6 M Long-Evans group 2
Feeding Condition	Fasted overnight until 4 hours postdose
Vehicle Formulation	Sterile water
Method of Administration	Oral
Dose (mg/kg)	200, single dose
Radionuclide	^{14}C
Specific Activity	135 μCi/mg
Sampling Time	1, 4, 8, 24, 72 and 144 hours

	Mean Tissue:Plasma Concentration Ratios Post Dosing											
	1 hour		4 hours		8 hours		24 hours		72 hours		144 hours	
Tissues/Organs	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Blood	0.762	0.057	0.829	0.041	NA	NA	NA	NA	NA	NA	NA	NA
Large Intestine Content	0.742	0.037	1.01	0.273	15.0	7.00	NA	NA	NA	NA	NA	NA
Kidney	2.45	0.388	2.10	0.431	2.62	NA	NA	NA	NA	NA	NA	NA
Liver	1.17	0.055	1.09	0.079	NA	NA	NA	NA	NA	NA	NA	NA
Renal Cortex	2.21	0.393	2.05	0.410	2.52	NA	NA	NA	NA	NA	NA	NA
Small Intestine Content	1.27	0.359	1.31	0.596	NA	NA	NA	NA	NA	NA	NA	NA
Cerebellum	0.068	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Cerebrum	0.066	0.001	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Additional Information	Data for SD rats only.											

F = female; M = male; NA= not available or not sampled.

5.2. RPV

5.2.1. TMC278-NC108: Tissue Distribution of [^{14}C]RPV in the Pigmented Rat after Oral Administration

Report Title	Study Type	Test Article	Report Number
Tissue Distribution of ^{14}C-R278474, as Studied by Whole-Body Autoradiography, in the Pigmented Male Rat After Single Oral Administration of ^{14}C-R278474 at 40 mg/kg	Tissue Distribution	[^{14}C]RPV	TMC278–NC108

Species	Rat (pigmented Long Evans)
Feeding Condition	Not fasted
Vehicle/Formulation	TMC278 base in PEG400/CA (10%)
Route	Oral (gavage)
Gender (M/F)/Number of Animals	M/5
Dose (mg/kg)	40
Radionuclide	^{14}C-TMC278
Specific Activity (kBq/mg)	233

Report Title	Study Type	Test Article	Report Number
Tissue Distribution of ^{14}C-R278474, as Studied by Whole-Body Autoradiography, in the Pigmented Male Rat After Single Oral Administration of ^{14}C-R278474 at 40 mg/kg	Tissue Distribution	[^{14}C]RPV	TMC278–NC108

Sampling Times (h)	**1**		**4**		**24**		**96**		**336**	
Tissues/Organs	**Conc. (µg eq./g)**	**Tissue/ Blood**	**Conc. (µg eq./g)**	**Tissue/ Blood**	**Conc. (µg eq./g)**	**Tissue/ Blood**	**Conc. (µg eq./g)**	**Tissue/ Blood**	**Conc. (µg eq./g)**	**Tissue/ Blood**
Adrenal gland	3.29	4.64	7.93	5.13	1.21	8.66	0.351	6.15	BLQ	-
Blood (LSC)	0.710	1.00	1.34	0.867	0.139	1.00	0.057	1.00	0.026	1.00
Blood (RLG)	0.708	1.00	1.55	1.00	BLQ	-	BLQ	-	BLQ	-
Bone	BLQ	-	0.348	0.225	BLQ	-	BLQ	-	BLQ	-
Bone marrow	1.10	1.55	2.00	1.29	BLQ	-	BLQ	-	BLQ	-

Report Title	Study Type	Test Article	Report Number
Tissue Distribution of ^{14}C-R278474, as Studied by Whole-Body Autoradiography, in the Pigmented Male Rat After Single Oral Administration of ^{14}C-R278474 at 40 mg/kg	Tissue Distribution	[^{14}C]RPV	TMC278–NC108

Sampling Times (h)	1		4		24		96		336	
Tissues/Organs	Conc. (µg eq./g)	Tissue/ Blood	Conc. (µg eq./g)	Tissue/ Blood	Conc. (µg eq./g)	Tissue/ Blood	Conc. (µg eq./g)	Tissue/ Blood	Conc. (µg eq./g)	Tissue/ Blood
Brain	0.506	0.715	0.981	0.634	BLQ	-	BLQ	-	BLQ	-
Brown fat	2.95	4.17	6.00	3.88	BLQ	-	BLQ	-	BLQ	-
Eye ball (LSC)	0.967	1.37	5.08	3.29	3.73	26.8	1.78	31.2	1.03	39.8
Heart	1.42	2.00	2.77	1.79	BLQ	-	BLQ	-	BLQ	-
Kidney	2.78	3.93	5.27	3.41	0.707	5.07	BLQ	-	BLQ	-
Liver	9.52	13.4	16.6	10.7	2.28	16.4	0.390	6.82	BLQ	-
Lung	1.03	1.45	3.54	2.29	BLQ	-	BLQ	-	BLQ	-
Meninges	1.84	2.60	5.80	3.75	1.73	12.4	1.57	27.5	1.21	46.6
Muscle	0.555	0.783	1.23	0.792	BLQ	-	BLQ	-	BLQ	-
Pancreas	2.20	3.11	4.23	2.73	BLQ	-	BLQ	-	BLQ	-
Prostate	0.865	1.22	2.07	1.34	BLQ	-	BLQ	-	BLQ	-
Skin pigmented	0.915	1.29	3.73	2.41	1.60	11.5	1.67	29.3	0.657	-
Skin white	1.10	1.55	2.93	1.89	0.761	5.47	0.470	8.24	BLQ	-
Spleen	NA	-	4.18	2.70	BLQ	-	BLQ	-	BLQ	-
Testis	0.306	0.433	1.25	0.807	BLQ	-	BLQ	-	BLQ	-
Thyroid	1.58	2.23	2.26	1.46	BLQ	-	BLQ	-	BLQ	-
Uveal tract	5.45	7.70	28.2	18.2	38.3	275	10.9	191	6.86	265
White fat	0.945	1.33	5.63	3.64	BLQ	-	BLQ	-	BLQ	-

Additional Information
Tissue/ blood concentration ratios were calculated preferably with blood total radioactivity levels as determined with RLG till 4 hours after dosing and as determined with LSC from 24 hours onwards.

Report Title	Study Type	Test Article	Report Number
Tissue Distribution of ^{14}C-R278474, as Studied by Whole-Body Autoradiography, in the Pigmented Male Rat After Single Oral Administration of ^{14}C-R278474 at 40 mg/kg	Tissue Distribution	[^{14}C]RPV	TMC278–NC108

Sampling Times (h)	**1, 4, 24, 96 and 336**	
Tissues/Organs	**AUC_{0-4h} (µg.h/g)**	**Tissue/Blood AUC_{0-4h} ratio**
Adrenal gland	18.5 (140 [a])	4.95 (6.76 [b])
Blood (LSC)	3.43 [c]	0.92
Blood (RLG)	3.74	1.00
Bone	NC [d]	-
Bone marrow	5.20	1.39
Brain	2.48	0.66
Brown fat	14.9	3.98
Eye ball (LSC)	9.55 (616 [e])	2.55 (20.5 [f])
Heart	6.98	1.87
Kidney	13.5 (58.9 [g])	3.61 (4.21 [h])
Liver	43.9 (265 [a])	11.7 (12.8 [b])
Lung	7.38	1.97
Meninges	12.4 (530 [c])	3.32 (17.6 [d])
Muscle	2.95	0.79
Pancreas	10.7	2.86
Prostate	4.83	1.29
Skin pigmented	7.42 (436 [c])	1.98 (14.5 [d])
Skin white	6.59 (82.2 [a])	1.76 (3.97 [b])
Spleen	-	-
Testis	2.48	0.66
Thyroid	6.55	1.75
Uveal tract	53.2 (4380 [e])	14.2 (146 [f])
White fat	10.3	2.75

Additional Information a AUC_{0-96h}; b calculated with AUC_{0-96h}; c AUC_{0-336h}; d calculated with AUC_{0-336h}
-: not applicable; CA: citric acid; LSC: liquid scintillation counting; PEG400: polyethylene glycol 400; RLG: radioluminography

5.2.2. TMC278–NC109: Tissue Distribution of [^{14}C]RPV in the Pregnant Rat after Oral Administration

Report Title	Study Type	Test Article	Report Number
Tissue Distribution and Placental Transfer of 14C-TMC278, as Studied by Whole-Body Autoradiography, in the Pregnant Sprague-Dawley Rat after Single Oral Administration at 40 mg/kg	Tissue Distribution	[^{14}C]RPV	TMC278–NC109

Species	Rat (Sprague Dawley, pregnant)
Feeding Condition	Not fasted
Vehicle/Formulation	TMC278 base in PEG400/CA (10%)
Route	Oral (gavage)
Gender (M/F)/Number of Animals	F/4
Dose (mg/kg)	40
Radionuclide	^{14}C-TMC278
Specific Activity (kBq/mg)	233

Report Title	Study Type	Test Article	Report Number
Tissue Distribution and Placental Transfer of 14C-TMC278, as Studied by Whole-Body Autoradiography, in the Pregnant Sprague-Dawley Rat after Single Oral Administration at 40 mg/kg	Tissue Distribution	[^{14}C]RPV	TMC278–NC109

Sampling Times (h)	1		4		8		24	
Tissues/Organs	Conc (µg eq./g)	Tissue/Blood	Conc (µg eq./g)	Tissue/Blood	Tissues/Organs	Conc. (µg eq./g)	Tissue/Blood	Conc. (µg eq./g)
Adrenal gland	4.52	3.59	9.76	3.47	7.37	4.05	1.75	17.0
Blood (LSC)	1.01	0.802	2.32	0.83	1.40	0.769	0.102	1.00
Blood (RLG)	1.26	1.00	2.81	1.00	1.82	1.00	BLQ	-
Brain	0.849	0.674	1.92	0.683	1.24	0.681	BLQ	-
Fat brown	3.55	2.82	7.29	2.59	6.22	3.42	BLQ	-
Fat white	1.37	1.09	8.18	2.91	8.12	4.46	BLQ	-
Fetus	0.627	0.498	1.87	0.665	1.19	0.654	BLQ	-
Heart	1.85	1.47	3.76	1.34	2.73	1.50	BLQ	-
Kidney	3.91	3.10	5.78	2.06	5.76	3.17	0.582	5.70
Lachrymal gland	3.87	3.07	9.56 [a]	3.40	7.48	4.11	BLQ	-
Liver	7.73	6.14	14.4	5.13	13.3	7.31	0.800	7.84
Lung	2.42	1.92	5.19	1.85	3.46	1.90	BLQ	-
Mammary gland	2.46	1.95	7.70	2.74	7.72	4.24	0.400	3.92
Muscle	0.643	0.510	1.39	0.495	1.14	0.626	BLQ	-
Ovary	NA [b]	-	5.90	2.10	4.03	2.21	BLQ	-
Pancreas	2.59	2.06	4.34	1.54	4.08	2.24	BLQ	-
Placenta	1.10	0.87	2.63	0.936	1.88	1.03	BLQ	-
Salivary gland	1.96	1.56	4.00	1.42	2.94	1.62	BLQ	-
Spleen	1.84	1.46	2.82	1.00	2.37	1.30	BLQ	-
Uterine epithelium	1.49	1.18	10.6	3.77	10.6	5.82	5.51	54.0
Uterus	1.18 [a]	0.937	2.63 [a]	0.936	2.36	1.30	0.303 [c]	3.00
Vagina	0.857	0.680	2.36	0.840	1.95	1.07	0.249 [a]	2.44

Additional Information : Tissue/ blood concentration ratios were calculated preferably with blood total radioactivity levels as determined with RLG till 8 hours after dosing and as determined with LSC at 24 hours.

a n=2; b NA: not analyzed due to flare effect of the high radioactive concentration in the formulation in stomach; c n=1

-: not applicable; BLQ: below limit of quantification (0.196 µg eq./g); CA: citric acid; LSC: liquid scintillation counting; PEG400: polyethylene glycol 400; RLG: radioluminography

Report Title	Study Type	Test Article	Report Number
Tissue Distribution and Placental Transfer of 14C-TMC278, as Studied by Whole-Body Autoradiography, in the Pregnant Sprague-Dawley Rat after Single Oral Administration at 40 mg/kg	Tissue Distribution	[^{14}C]RPV	TMC278–NC109

Sampling Times (h)	**1, 4, 8 and 24**	
Tissues/Organs	**AUC_{0-4h} (µg.h/g)**	**Tissue/Blood AUC_{0-4h} ratio**
Adrenal gland	57.7 (120[a])	3.6 (5.8[c])
Blood (LSC)	12.8 (20.7[a])	0.81
Blood (RLG)	15.9	1.0
Brain	10.8	0.68
Fat brown	45.0	2.8
Fat white	47.6	3.0
Fetus	10.1	0.64
Heart	22.2	1.4
Kidney	39.6 (75.7[a])	2.5 (3.7[c])
Lachrymal gland	56.0	3.5
Liver	92.3 (163[a])	5.8 (7.9[c])
Lung	29.7	1.9
Mammary gland	47.3 (86.9[a])	3.0 (4.2[c])
Muscle	8.41	0.53
Ovary	31.4[b]	2.0
Pancreas	28.5	1.8
Placenta	15.1	0.95
Salivary gland	23.7	1.5
Spleen	18.3	1.2
Uterine epithelium	61.2 (186[a])	3.8 (9.0[c])
Uterus	16.3 (32.3[a])	1.0 (1.6[c])
Vagina	13.9 (27.1[a])	0.87 (1.3[c])

CA: citric acid; LSC: liquid scintillation counting; PEG400: polyethylene glycol 400; RLG: radioluminography
a AUC_{0-24h}; b AUC was calculated with concentration versus time data at 4 and 8 hours. For this tissue, no data were available at 1 hour post dose; c AUC_{0-24h} ratio calculated with blood (LSC) = 20.7 µg eq.h/g)

5.3. TAF

5.3.1. AD-120-2011: Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]TAF in Mouse Following Oral Administration

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Species	CD-1 Mice
Sex (M/F)/No. of Animals	M/30
Method of Administration	Oral gavage
Dose (mg/kg/day)	100
Feeding Condition	Not fasted
Specific Activity	300 µCi/kg
Radionuclide	Carbon-14
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample	Plasma/Blood
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

Tabulated PK Results (Mean):

Sample Type	Plasma	Blood
T_{max} (h)	0.25	0.50
C_{max} (ng eq/g)	24500	23100
$t_{½}$ (h)	15.8	45.0
AUC_{0-t} (ng eq•h/g)	108675	514848
$AUC_{0-\infty}$ (ng eq•h/g)	111574	NA

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Species	CD-1 Mice
Sex (M/F) No. of Animals	M/30
Method of Administration	Oral gavage
Dose (mg/kg)	100
Feeding Condition	Not fasted
Radionuclide	Carbon-14
Specific Activity	300 µCi/kg
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

	Mean Concentrations Postdosing (ng Equivalents [^{14}C]TAF/g)				
Time Point	**Brain**	**Heart**	**Kidney**	**Liver**	**Nasal turbinates**
1 h	604	7660	106000	519000	5680
4 h	183	2440	67500	394000	1750
12 h	198	4290	47900	296000	1870
24 h	219	4870	26900	194000	1540
48 h	159	3550	13400	83600	1170
72 h	88.9	1460	5820	55700	546

F = female; M = male; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Species	CD-1 Mice
Sex (M/F) / No. of Animals	M/9
Method of Administration:	Oral gavage
Dose (mg/kg):	100
Feeding Condition:	Not fasted
Radionuclide:	Carbon-14
Specific Activity:	300 µCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sampling Time:	0.5, 1, 3, 8, 12, 24, 48, 96, and 168 hours postdose
Analyte/Assay:	[^{14}C]TAF/Quantitative whole body autoradiography

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Blood and Tissue radioactivity:	**Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g)**								
	Animal Number (Sacrifice Time)								
Tissue	**A18225 0.5h**	**A18226 1 h**	**A18227 3 h**	**A18228 8 h**	**A18229 12 h**	**A18230 24 h**	**A18231 48 h**	**A18232 96 h**	**A18233 168 h**
Adrenal gland(s)	9920	27500	6450	5420	3010	3600	2550	1530	669
Bile	60300	127000	115000	NR	65900	12900	12200	5940	NR
Blood	14600	14500	5280	8640	4380	6100	4100	2710	1000
Bone	1300	2420	1230	1140	1010	525	BLQ	372	ND
Bone marrow	4730	6610	2030	1670	2040	2120	1190	1140	513
Brain cerebellum	569	1940	395	BLQ	BLQ	BLQ	BLQ	ND	ND
Brain cerebrum	768	1430	BLQ	BLQ	379	BLQ	BLQ	ND	ND
Brain medulla	420	833	BLQ	BLQ	BLQ	BLQ	BLQ	ND	ND
Brain olfactory lobe	1120	2890	464	398	461	BLQ	BLQ	ND	ND
Cecum	3440	6960	90500	63200	19100	8310	2290	2540	1310
Diaphragm	40600	167000	46600	82900	26600	28300	19000	10300	3320
Epididymis	3600	22300	1310	508	540	864	BLQ	ND	ND
Esophagus	38400	90800	57300	114000	20100	8660	8660	5860	933
Exorbital lacrimal gland	3600	5460	1400	1840	1250	1290	748	729	364
Eye lens	967	2980	508	BLQ	BLQ	BLQ	BLQ	BLQ	ND
Eye uveal tract	4180	3690	754	488	947	779	BLQ	ND	ND
Eye(s)	1880	2660	539	312	418	363	BLQ	BLQ	ND
Fat (abdominal)	1250	1170	968	757	512	787	BLQ	ND	ND
Fat (brown)	3070	5590	2530	3790	2060	3030	1920	1060	BLQ
Gall bladder	335000	216000	108000	NR	68100	37100	20900	29200	NR
Harderian gland	4240	6500	1530	2050	2170	1390	764	1210	487
Intra-orbital lacrimal gland	7630	3580	1900	1190	2280	1500	NR	ND	442
Kidney cortex	82300	89000	74000	37400	30000	23100	10500	7300	2830
Kidney medulla	65000	70000	54800	29600	18100	12800	7070	4430	1670

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Blood and Tissue radioactivity:	**Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g)**								
	Animal Number (Sacrifice Time)								
Tissue	**A18225 0.5h**	**A18226 1 h**	**A18227 3 h**	**A18228 8 h**	**A18229 12 h**	**A18230 24 h**	**A18231 48 h**	**A18232 96 h**	**A18233 168 h**
Kidney(s)	84800	86100	68900	3370	25900	19900	9090	6080	2300
Large intestine	6680	8090	7730	96100	22500	6770	3100	2200	607
Liver	282000	447000	290000	295000	197000	164000	77000	44600	19000
Lung(s)	13200	23900	4000	8900	10000	11500	6890	5220	1820
Lymph node(s)	2190	7750	1680	1690	1760	2280	1470	2090	468
Muscle	1680	2100	466	522	768	579	363	366	BLQ
Myocardium	7570	11700	2380	4100	4600	4620	3430	3500	905
Nasal turbinates	2720	6440	1290	1170	983	1190	519	709	370
Pancreas	6130	14500	2950	2970	3540	2880	1760	1970	917
Pituitary gland	2910	7440	1280	1390	2510	1300	663	718	BLQ
Preputial gland	1900	2580	635	390	708	503	BLQ	ND	ND
Prostate gland	5500	2880	2150	16500	3060	2590	427	440	ND
Salivary gland(s)	5390	11500	1880	2210	2420	2160	1130	907	511
Seminal vesicle(s)	1790	3410	957	27000	852	620	462	BLQ	ND
Skin (pigmented)	8520	5580	1510	939	674	715	368	BLQ	ND
Small intestine	8770	74500	88100	54900	14100	4040	3400	3520	1610
Spinal cord	757	1210	BLQ	437	BLQ	BLQ	BLQ	ND	ND
Spleen	6100	12900	3850	4700	5460	6270	4020	3040	1430
Stomach	77600	39300	26600	49700	5460	5940	5870	1760	571
Stomach mucosa	96900	46900	19800	44000	5280	3660	5910	2550	638
Stomach wall	48600	24500	26600	24700	7690	22700	4230	3940	378
Testis(es)	1160	1490	774	BLQ	BLQ	319	ND	ND	ND
Thymus	2510	6140	914	1300	903	1190	808	789	BLQ
Thyroid	7120	12100	2830	2290	1200	4600	3200	3500	1530
Urinary bladder	ND	174000	85600	ND	5800	10300	9240	2800	924
Urine	1790000	413000	200000	351000	36800	24700	14600	327	BLQ

BLQ = below the limit of quantitation (< 311 ng equivalents [^{14}C]GS-7340/g); F = female; M = male; ND = not detectable; NR = not represented; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Methods:

Species	C57 Black Mice
Sex (M/F) / No. of Animals	M/9
Method of Administration:	Oral gavage
Dose (mg/kg):	100
Feeding Condition:	Not fasted
Radionuclide:	Carbon-14
Specific Activity:	300 µCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sampling Time:	0.5, 1, 3, 12, 24, 96, 168, 240, and 336 hours postdose
Analyte/Assay:	[^{14}C]TAF/Quantitative whole body autoradiography

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Blood and Tissue radioactivity:	**Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)**								
Tissue	**A19068 0.5h**	**A19069 1 h**	**A19070 3 h**	**A19071 12 h**	**A19072 24 h**	**A19073 96 h**	**A19074 168 h**	**A19075 240 h**	**A19076 336 h**
Adrenal gland(s)	34000	26400	20400	7750	10400	2710	2310	1610	1430
Bile	198000	136000	150000	40300	26600	3620	1020	2360	2160
Blood	16700	9270	7910	4170	3780	1100	788	675	BLQ
Bone	11200	4490	4900	2430	1770	ND	ND	ND	ND
Bone marrow	24300	19000	16100	6440	6470	ND	ND	ND	ND
Brain cerebellum	611	590	BLQ	ND	ND	ND	ND	ND	ND
Brain cerebrum	1240	661	BLQ	ND	ND	ND	ND	ND	ND
Brain medulla	886	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Brain olfactory lobe	3790	1730	1640	688	707	ND	ND	ND	ND
Cecum	25400	95400	ND	50500	10500	BLQ	ND	ND	ND
Diaphragm	60300	103000	114000	27500	25900	2340	2360	1830	1410
Epididymis	10800	4190	4990	2120	2150	ND	ND	ND	ND
Esophagus	76100	81300	48900	27900	8540	1540	1660	723	ND
Exorbital lacrimal gland	18100	12000	11800	4910	3530	1050	1010	940	ND
Eye lens	4310	1870	1110	527	BLQ	ND	ND	ND	ND
Eye uveal tract	13400	12200	11600	4740	6140	ND	ND	ND	ND
Eye(s)	5000	2920	2440	1040	1060	ND	ND	ND	ND
Fat (abdominal)	5460	6820	2140	4180	1700	6410	BLQ	ND	ND
Fat (brown)	19200	18700	14500	10800	8660	2420	1190	812	569
Gall bladder	163000	379000	275000	94000	39800	6580	4130	ND	ND
Harderian gland	18500	14600	13600	6200	4580	861	ND	ND	ND
Intra-orbital lacrimal gland	NR	NR	NR	NR	3760	ND	ND	ND	ND
Kidney cortex	137000	125000	104000	58000	34500	5660	5350	4300	2530
Kidney medulla	125000	94700	80000	40200	19400	3640	2980	2620	1820
Kidney(s)	132000	107000	89600	47500	29500	4820	4540	3550	2240
Large intestine	25700	17300	32300	74200	35000	2930	1530	785	BLQ

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Blood and Tissue radioactivity:	**Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)**								
Tissue	**A19068 0.5h**	**A19069 1 h**	**A19070 3 h**	**A19071 12 h**	**A19072 24 h**	**A19073 96 h**	**A19074 168 h**	**A19075 240 h**	**A19076 336 h**
Liver	488000	490000	385000	282000	118000	21900	14200	9840	7510
Lung(s)	32300	32500	26500	18100	13500	4440	4260	3330	2120
Lymph node(s)	17900	14600	12600	8020	10900	ND	ND	ND	ND
Muscle	8780	4140	3190	2010	3900	753	982	650	491
Myocardium	23200	13500	13600	8470	9510	2490	2170	2070	1540
Nasal turbinates	10600	2980	3550	2390	1920	799	ND	ND	ND
Pancreas	34900	26900	31500	15400	13900	1780	1940	1650	1220
Pituitary gland	17400	8440	6590	850	ND	ND	ND	ND	ND
Preputial gland	11500	6390	6130	2240	1970	ND	ND	ND	ND
Prostate gland	ND	8630	4840	8620	ND	ND	ND	ND	ND
Salivary gland(s)	36200	24200	27200	9910	12200	1150	1080	899	522
Seminal vesicle(s)	5540	3530	2470	2150	825	BLQ	ND	ND	ND
Skin (pigmented)	11600	5930	3350	1180	1340	ND	ND	ND	ND
Small intestine	26900	56100	28000	14100	11300	5030	ND	ND	ND
Spinal cord	1950	742	BLQ	BLQ	ND	ND	ND	ND	ND
Spleen	35300	29600	29100	16600	12200	2570	2560	1550	978
Stomach	60500	70600	29200	7900	6730	1610	1500	1230	859
Stomach mucosa	ND	ND	ND	ND	ND	ND	ND	ND	ND
Stomach wall	ND	ND	ND	ND	ND	ND	ND	ND	ND
Testis(es)	2640	1760	1070	512	752	ND	ND	ND	ND
Thymus	12400	8190	7190	3440	3410	BLQ	ND	ND	ND
Thyroid	40700	32700	29000	NR	10900	4840	4150	3160	2620
Urinary bladder	ND	138000	49100	12000	6290	1290	ND	ND	ND
Urine	1170000	626000	128000	135000	18400	495	ND	ND	ND

BLQ = below the limit of quantitation (< 490 ng equivalents [^{14}C]GS-7340/g); F = female; M = male; ND = not detectable; NR = not represented; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Species	CD-1 Mice
Sex (M/F) / No. of Animals	M/4
Method of Administration:	Oral gavage
Dose (mg/kg):	100
Feeding Condition:	Not fasted
Radionuclide:	Carbon-14
Specific Activity:	300 µCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/ Liquid Scintillation Counter

	Cumulative Excretion of Radioactivity (% of dose)			
Time Point	**Urine**	**Feces**	**Cage Rinse**	**Total**
0-12 h	9.75	NA	NA	9.75
0-24 h	19.3	31.1	3.95	54.4
0-48 h	24.6	36.1	4.91	65.6
0-72 h	25.9	37.6	5.50	69.0
0-96 h	26.7	39.4	6.06	72.2
0-120 h	27.1	40.4	6.40	73.9
0-144 h	27.4	41.0	6.65	75.1
0-168 h	27.7	41.3	NA	69.0

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide

5.3.2. AD-120-2020: Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]TAF in Rat Following Oral Administration

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Methods:

Species	Sprague-Dawley Rats
Sex (M/F)/No. of Animals	M/15
Method of Administration	Oral gavage
Dose (mg/kg/day)	5
Feeding Condition	Fasted
Specific Activity	100 µCi/mg
Radionuclide	Carbon-14
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample	Plasma/Blood
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

Tabulated PK Results (Mean):

Sample Type	Plasma	Blood
T_{max} (h)	0.25	0.25
C_{max} (ng eq/g)	1110	603
t½ (h)	14.1	21.5
AUC_{0-t} (ng eq•h/g)	3591	2432
$AUC_{0-\infty}$ (ng eq•h/g)	3870	2588

F = female; M = male; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Methods:

Species	Sprague-Dawley Rats
Sex (M/F) / No. of Animals	M/9
Method of Administration:	Oral gavage
Dose (mg/kg):	5
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	100 μCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sampling Time:	0.25, 1, 4, 8, 12, 24, 48, 96, and 168 hours postdose
Analyte/Assay:	[^{14}C]TAF/Quantitative whole body autoradiography

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Blood and Tissue radioactivity:

	Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g)								
	Animal Number (Sacrifice Time)								
Tissue	B35426 0.25h	B35427 1 h	B35428 4 h	B35429 8 h	B35430 12 h	B35431 24 h	B35432 48 h	B35433 96 h	B35434 168 h
Adrenal gland(s)	181	129	BLQ	ND	ND	ND	ND	ND	ND
Arterial wall	817	299	118	ND	ND	ND	ND	ND	ND
Bile	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blood	1070	334	138	76.6	83.1	ND	ND	ND	ND
Bone	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Bone marrow	233	125	72.6	BLQ	BLQ	ND	ND	ND	ND
Brain cerebellum	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain cerebrum	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain medulla	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain olfactory lobe	45.7	BLQ	ND	ND	ND	ND	ND	ND	ND
Bulbo-urethral gland	396	177	236	BLQ	ND	ND	ND	ND	ND
Cecum	218	132	541	313	NR	323	261	ND	ND
Diaphragm	210	145	52.9	BLQ	ND	ND	ND	ND	ND
Epididymis	249	101	BLQ	BLQ	ND	ND	ND	ND	ND
Esophagus	341	222	187	79.8	58.9	ND	ND	ND	ND
Exorbital lacrimal gland	234	101	51.2	ND	ND	ND	ND	ND	ND
Eye lens	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Eye uveal tract	409	187	78.4	ND	ND	ND	ND	ND	ND
Eye vitreous humor	84.9	89.3	BLQ	ND	ND	ND	ND	ND	ND
Eye(s)	86.1	92.7	BLQ	ND	ND	ND	ND	ND	ND
Fat (abdominal)	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND
Fat (brown)	200	97.1	48.2	BLQ	55.7	ND	ND	ND	ND
Harderian gland	98.6	52.4	BLQ	ND	ND	ND	ND	ND	ND
Intra-orbital lacrimal gland	250	118	BLQ	ND	ND	ND	ND	ND	ND
Kidney cortex	10700	12400	11800	6410	8300	2010	677	70.3	ND

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Blood and Tissue radioactivity:

	Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g)								
	Animal Number (Sacrifice Time)								
Tissue	B35426 0.25h	B35427 1 h	B35428 4 h	B35429 8 h	B35430 12 h	B35431 24 h	B35432 48 h	B35433 96 h	B35434 168 h
Kidney medulla	8240	5040	2800	1210	1010	317	161	BLQ	ND
Kidney(s)	9520	8710	8250	4720	4590	1380	511	52.0	ND
Large intestine	364	140	55.8	BLQ	ND	ND	ND	ND	ND
Liver	6730	6730	4010	2410	3570	1090	751	145	195
Lung(s)	592	211	81.1	ND	ND	ND	ND	ND	ND
Lymph node(s)	318	ND	ND	ND	ND	ND	ND	ND	ND
Muscle	101	BLQ	ND	ND	ND	ND	ND	ND	ND
Myocardium	361	136	58.6	BLQ	ND	ND	ND	ND	ND
Nasal turbinates	123	83.8	52.8	ND	ND	ND	ND	ND	ND
Pancreas	215	120	68.9	46.1	52.0	ND	ND	ND	ND
Pituitary gland	335	116	50.5	BLQ	76.2	ND	ND	ND	ND
Preputial gland	140	60.1	BLQ	ND	ND	ND	ND	ND	ND
Prostate gland	134	88.6	117	BLQ	BLQ	ND	ND	ND	ND
Salivary gland(s)	292	119	BLQ	ND	ND	ND	ND	ND	ND
Seminal vesicle(s)	62.7	ND	ND	ND	ND	ND	ND	ND	ND
Skin (nonpigmented)	393	123	BLQ	ND	ND	ND	ND	ND	ND
Small intestine	479	500	364	284	86.5	171	58.3	ND	ND
Spinal cord	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Spleen	147	105	59.0	BLQ	62.8	BLQ	ND	ND	ND
Stomach	475	126	66.6	ND	ND	ND	ND	ND	ND
Testis(es)	98.2	50.9	BLQ	ND	ND	ND	ND	ND	ND

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Blood and Tissue radioactivity:

	Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g)								
	Animal Number (Sacrifice Time)								
Tissue	**B35426 0.25h**	**B35427 1 h**	**B35428 4 h**	**B35429 8 h**	**B35430 12 h**	**B35431 24 h**	**B35432 48 h**	**B35433 96 h**	**B35434 168 h**
Thymus	91.2	68.1	BLQ	ND	ND	ND	ND	ND	ND
Thyroid	324	147	63.6	ND	ND	ND	ND	ND	ND
Tooth pulp	646	228	84.4	ND	ND	ND	ND	ND	ND
Urinary bladder	925	205	ND	BLQ	1050	BLQ	ND	ND	ND
Urine	112000	34400	51100	3260	14500	988	645	88.3	ND

BLQ = below the limit of quantitation (< 45.6 ng equivalents [^{14}C]GS-7340/g); F = female; M = male; ND = not detectable; NR = not represented (tissue not present in section); TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Methods:

Species	Long Evans rats
Sex (M/F) / No. of Animals	M/9
Method of Administration:	Oral gavage
Dose (mg/kg):	5
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	100 μCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sampling Time:	0.25, 1, 4, 8, 12, 24, 48, 96, and 168 hours postdose
Analyte/Assay:	[^{14}C]TAF/Quantitative whole body autoradiography

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Blood and Tissue radioactivity:

Tissue	Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)								
	B35435 0.25h	B35436 1 h	B35437 4 h	B35438 8 h	B35439 12 h	B35440 24 h	B35441 48 h	B35442 96 h	B35443 168 h
Adrenal gland(s)	353	115	48.5	ND	ND	ND	ND	ND	ND
Arterial wall	1350	270	90.4	93.8	ND	ND	ND	ND	ND
Bile	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blood	1260	221	116	125	117	ND	ND	ND	ND
Bone	50.4	BLQ	ND	ND	ND	ND	ND	ND	ND
Bone marrow	311	84.9	BLQ	62.4	BLQ	ND	ND	ND	ND
Brain cerebellum	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain cerebrum	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain medulla	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain olfactory lobe	57.2	51.0	ND	ND	ND	ND	ND	ND	ND
Bulbo-urethral gland	831	209	ND	ND	ND	ND	ND	ND	ND
Cecum	603	118	889	462	362	494	101	BLQ	ND
Diaphragm	353	124	55.8	BLQ	ND	ND	ND	ND	ND
Epididymis	516	79.3	BLQ	58.4	ND	ND	ND	ND	ND
Esophagus	923	218	186	637	65.8	ND	ND	ND	ND
Exorbital lacrimal gland	353	56.1	BLQ	BLQ	ND	ND	ND	ND	ND
Eye lens	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND
Eye uveal tract	555	89.5	59.4	BLQ	ND	ND	ND	ND	ND
Eye vitreous humor	139	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Eye(s)	150	BLQ	BLQ	BLQ	ND	ND	ND	ND	ND
Fat (abdominal)	70.0	BLQ	ND	ND	ND	ND	ND	ND	ND

Blood and Tissue radioactivity:	**Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)**								
Tissue	**B35435 0.25h**	**B35436 1 h**	**B35437 4 h**	**B35438 8 h**	**B35439 12 h**	**B35440 24 h**	**B35441 48 h**	**B35442 96 h**	**B35443 168 h**
Fat (brown)	232	BLQ	ND	ND	ND	ND	ND	ND	ND
Harderian gland	209	BLQ	ND	ND	ND	ND	ND	ND	ND
Intra-orbital lacrimal gland	402	53.4	49.1	ND	ND	ND	ND	ND	ND
Kidney cortex	8000	8890	6980	7360	5150	2440	570	65.6	ND
Kidney medulla	6900	3670	655	1920	757	367	117	BLQ	ND
Kidney(s)	7750	7570	5160	5260	3000	1310	390	48.1	ND
Large intestine	548	91.5	474	97.5	133	132	575	166	ND
Liver	10300	7800	7710	6670	5610	1380	671	221	139
Lung(s)	854	145	67.2	76.5	66.3	BLQ	ND	ND	ND
Lymph node(s)	422	94.9	ND	ND	ND	ND	ND	ND	ND
Muscle	115	BLQ	ND	ND	ND	ND	ND	ND	ND
Myocardium	512	46.6	BLQ	72.7	BLQ	ND	ND	ND	ND
Nasal turbinates	201	71.9	BLQ	ND	ND	ND	ND	ND	ND
Pancreas	274	82.1	53.5	71.0	ND	ND	ND	ND	ND
Pituitary gland	434	53.1	ND	ND	ND	ND	ND	ND	ND
Preputial gland	247	67.9	ND	ND	ND	ND	ND	ND	ND
Prostate gland	247	BLQ	49.9	ND	ND	ND	ND	ND	ND
Salivary gland(s)	368	85.3	BLQ	BLQ	BLQ	ND	ND	ND	ND
Seminal vesicle(s)	67.7	BLQ	ND	ND	ND	ND	ND	ND	ND
Skin (nonpigmented)	526	71.8	BLQ	ND	ND	ND	ND	ND	ND
Skin (pigmented)	623	78.9	BLQ	ND	ND	ND	ND	ND	ND
Small intestine	530	379	122	278	220	98.6	BLQ	ND	ND
Spinal cord	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND
Spleen	231	83.1	58.3	75.8	60.7	BLQ	BLQ	ND	ND
Stomach	682	113	71.4	69.9	159	BLQ	BLQ	ND	ND
Testis(es)	157	BLQ	BLQ	BLQ	BLQ	ND	ND	ND	ND
Thymus	181	BLQ	BLQ	BLQ	ND	ND	ND	ND	ND

Blood and Tissue radioactivity:	Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)								
	B35435	B35436	B35437	B35438	B35439	B35440	B35441	B35442	B35443
Tissue	0.25h	1 h	4 h	8 h	12 h	24 h	48 h	96 h	168 h
Thyroid	412	59.1	ND	ND	ND	ND	ND	ND	ND
Tooth pulp	793	194	78.7	89.3	57.0	NR	ND	ND	ND
Urinary bladder	352	155	125	89.1	45.9	48.3	BLQ	ND	ND
Urine	50200	61000	2280	6540	1140	1500	109	BLQ	ND

BLQ = below the limit of quantitation (< 45.6 ng equivalents [^{14}C]GS-7340/g); F = female; M = male; ND = not detectable; NR = not represented (tissue not present in section); TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Methods:

Species	Sprague-Dawley rats
Sex (M/F) / No. of Animals	M/3
Method of Administration:	Oral gavage
Dose (mg/kg):	5
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	100 µCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/ Liquid Scintillation Counter

	Cumulative Excretion of Radioactivity (% of dose)			
Time Point	**Urine**	**Feces**	**Cage Rinse**	**Total**
0-8 h	14.6 ± 3.78	NA	NA	14.6 ± 3.78
0-24 h	19.8 ± 4.96	66.8 ± 6.14	1.22 ± 0.16	87.9 ± 1.06
0-48 h	21.5 ± 5.45	71.1 ± 4.73	1.46 ± 0.08	94.1 ± 0.71
0-72 h	21.8 ± 5.45	71.6 ± 4.50	1.56 ± 0.15	95.0 ± 1.01
0-96 h	22.0 ± 5.48	71.8 ± 4.52	1.67 ± 0.26	95.4 ± 0.97
0-120 h	22.1 ± 5.47	71.8 ± 4.51	1.70 ± 0.28	95.6 ± 0.90
0-144 h	22.1 ± 5.47	71.9 ± 4.51	1.71 ± 0.28	95.7 ± 0.95
0-168 h	22.2 ± 5.42	71.9 ± 4.49	NA	94.1 ± 0.93

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Methods:

Species	Bile Duct-Cannulated SD rats
Sex (M/F) / No. of Animals	M/5
Method of Administration:	Oral gavage
Dose (mg/kg):	5
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	100 µCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/ Liquid Scintillation Counter

	Cumulative Excretion of Radioactivity (% of dose)				
Time Point	**Urine**	**Feces**	**Bile**	**Cage Rinse**	**Total**
0-2 h	NA	NA	1.81 ± 0.76	NA	1.81 ± 0.76
0-4 h	NA	NA	1.93 ± 0.76	NA	1.93 ± 0.76
0-6 h	NA	NA	1.97 ± 0.77	NA	1.97 ± 0.77
0-8 h	15.3 ± 1.82	NA	2.00 ± 0.78	NA	17.3 ± 2.18
0-12 h	NA	NA	2.04 ± 0.79	NA	2.04 ± 0.80
0-24 h	21.1 ± 3.42	56.7 ± 15.3	2.09 ± 0.81	0.91 ± 0.17	80.7 ± 11.4
0-48 h	22.7 ± 3.95	70.9 ± 7.10	2.11 ± 0.82	1.14 ± 0.21	96.8 ± 2.58
0-72 h	23.0 ± 4.06	72.3 ± 6.02	2.11 ± 0.82	1.28 ± 0.27	98.6 ± 1.49
0-96 h	23.1 ± 4.11	72.4 ± 5.95	2.11 ± 0.82	1.33 ± 0.29	99.0 ± 1.40
0-120 h	23.2 ± 4.12	72.5 ± 5.96	2.11 ± 0.82	1.35 ± 0.29	99.1 ± 1.40
0-144 h	23.2 ± 4.13	72.5 ± 5.96	2.11 ± 0.82	1.37 ± 0.30	99.2 ± 1.49
0-168 h	23.2 ± 4.15	72.6 ± 5.96	2.11 ± 0.82	NA	97.9 ± 1.35

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide

5.3.3. AD-120-2009: Distribution of [^{14}C]TAF in Dog Following Oral Administration

Report Title	Study Type	Test Article	Report Number
Absorption and Distribution of [^{14}C]GS-7340 Following Single and Multiple Oral Doses to Dogs	Distribution	[^{14}C]TAF	AD-120-2009

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/10
Method of Administration:	Oral gavage
Dose Administration:	Nonradiolabeled test article was dosed for 4 days followed by a single dose of radiolabeled test article on Day 5
Dose (mg/kg/day):	15
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	10 µCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/ Liquid Scintillation Counter

	Mean Concentration (ng equivalents [^{14}C]GS-7340/g)	
Time Point	**Blood**	**Plasma**
1 h	2220	3180
2 h	1290	1750
6 h	811	783
12 h	449	363
24 h	340	277

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide
Note: Mean concentrations were calculated from 2 animals/timepoint

Report Title	Study Type	Test Article	Report Number
Absorption and Distribution of [^{14}C]GS-7340 Following Single and Multiple Oral Doses to Dogs	Distribution	[^{14}C]TAF	AD-120-2009

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/10
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	10 µCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/ Liquid Scintillation Counter

	Mean Concentration (ng equivalents [^{14}C]GS-7340/g)	
Time Point	**Blood**	**Plasma**
1 h	485	778
2 h	1280	1890
6 h	401	355
12 h	295	262
24 h	201	172

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide
Note: Mean concentrations were calculated from 2 animals/timepoint

Report Title	Study Type	Test Article	Report Number
Absorption and Distribution of [^{14}C]GS-7340 Following Single and Multiple Oral Doses to Dogs	Distribution	[^{14}C]TAF	AD-120-2009

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/10
Method of Administration:	Oral gavage
Dose Administration:	Nonradiolabeled test article was dosed for 4 days followed by a single dose of radiolabeled test article on Day 5
Dose (mg/kg/day):	15
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	10 μCi/kg
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

	Concentrations (ng Equivalents [^{14}C]GS-7340/g)					
Tint Point	**Bone**	**Heart**	**Kidney(s)**	**Liver**	**Lung**	**Thyroid**
1 h	2390	3450	45500	74900	8660	15200
2 h	4410	4420	89300	105000	13000	26500
6 h	5600	5310	162000	109000	15000	30500
12 h	4350	4330	144000	84600	9640	21600
24 h	2990	3970	107000	78100	8610	16800

F = female; M = male; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Absorption and Distribution of [^{14}C]GS-7340 Following Single and Multiple Oral Doses to Dogs	Distribution	[^{14}C]TAF	AD-120-2009

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/10
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	10 μCi/kg
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

	Concentrations (ng Equivalents [^{14}C]GS-7340/g)					
Time Point	**Bone**	**Heart**	**Kidney(s)**	**Liver**	**Lung**	**Thyroid**
1 h	476	776	17000	47900	2480	2960
2 h	2440	2750	82500	97600	10200	12700
6 h	2600	3070	99700	97000	8870	15600
12 h	2220	2820	91800	78300	6970	16800
24 h	1100	1990	88500	64100	5260	4900

F = female; M = male; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Absorption and Distribution of [^{14}C]GS-7340 Following Single and Multiple Oral Doses to Dogs	Distribution	[^{14}C]TAF	AD-120-2009

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/10
Method of Administration:	Oral gavage
Dose Administration:	Nonradiolabeled test article was dosed for 4 days followed by a single dose of radiolabeled test article on Day 5
Dose (mg/kg/day):	15
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	10 μCi/kg
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

	Percent of Radioactive Dose (% of Dose)						
Time Point	**Blood**	**Bone**	**Heart**	**Kidney(s)**	**Liver**	**Lung**	**Thyroid**
1 h	0.64	1.48	0.19	1.39	15.4	0.50	0.013
2 h	0.37	2.70	0.24	2.78	23.1	0.75	0.019
6 h	0.23	3.45	0.26	4.17	19.4	0.87	0.020
12 h	0.13	2.69	0.24	3.93	18.3	0.60	0.011
24 h	0.10	1.85	0.22	2.76	15.0	0.50	0.008

F = female; M = male; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Absorption and Distribution of [^{14}C]GS-7340 Following Single and Multiple Oral Doses to Dogs	Distribution	[^{14}C]TAF	AD-120-2009

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/10
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	10 μCi/kg
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

	Percent of Radioactive Dose (% of Dose)						
Tint Point	**Blood**	**Bone**	**Heart**	**Kidney(s)**	**Liver**	**Lung**	**Thyroid**
1 h	0.14	0.30	0.05	0.60	8.78	0.19	0.002
2 h	0.37	1.51	0.17	2.84	19.6	0.62	0.009
6 h	0.12	1.61	0.17	3.46	18.6	0.50	0.009
12 h	0.08	1.37	0.15	3.02	18.2	0.44	0.012
24 h	0.06	0.67	0.10	2.79	13.9	0.34	0.003

F = female; M = male; TAF = tenofovir alafenamide

5.3.4. D990173-BP: Distribution of [^{14}C]TAF in Dog Following Oral Administration

Report Title	Study Type	Test Article	Report Number
Analysis of Data from Oread Biosafety Study G545 "Tissue Distribution of [^{14}C]GS-7340 in Beagle Dogs Following Oral Administration"	Distribution	[^{14}C]TAF	D990173-BP

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/2
Method of Administration:	Oral gavage
Dose (mg/kg):	18.1
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	31.7 µCi/kg
Vehicle Formulation	50 mM citric acid
Sampling Time:	24 hours postdose
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

Report Title	Study Type	Test Article	Report Number
Analysis of Data from Oread Biosafety Study G545 "Tissue Distribution of [^{14}C]GS-7340 in Beagle Dogs Following Oral Administration"	Distribution	[^{14}C]TAF	D990173-BP

Distribution of Radioactivity

Tissue/Fluid	Percent of Total Dose (%)	Percent of Recovered Dose[a] (%)	Concentration (µg eq TFV/g)
Liver	16.5	26.0	52.9
Kidney	3.78	5.98	80.2
Mesenteric Lymph Nodes	0.04	0.06	6.88
Spleen	0.17	0.27	8.13
Femoral Bone	0.00	0.00	0.28
Bone Marrow	0.00	0.00	2.05
Stomach	0.26	0.41	2.68
Jejunum	0.79	1.26	4.16
Duodenum	0.44	0.70	8.77
Ileum	0.16	0.26	4.61
large Intestine	2.66	4.28	47.2
Bile	0.23	0.35	40.5
Feces	0.19	0.15	480
Stomach Content	0.04	0.06	0.91
Small Intestine Content	1.25	2.00	11.3
Large Intestine Content	20.4	32.4	155
Urine	14.7	23.1	25.2
Plasma	0.00	0.00	0.20
PBMCs	0.09	0.14	63.2[b]

F = female; M = male; TAF = tenofovir alafenamide; TFV = tenofovir
a Selected tissues reported; b Estimated based on the mean reported single cell volume of 0.2 picoliters

6. PHARMACOKINETICS: PLASMA PROTEIN BINDING

6.1. FTC

6.1.1. TBZZ/93/0025: Protein Binding of FTC in Mouse, Rabbit, Monkey and Human Plasma

Report Title	Study Type	Test Article	Report Number
Protein Binding of 524W91 in Human, Monkey, Mouse, and Rabbit Plasma	Plasma Protein Binding	FTC	TBZZ/93/0025

Study system: In vitro
Target Entity, Test System, and Methods: Plasma, Equilibrium dialysis

Species	FTC concentration		% Bound
	µg/mL	µM	
Human	0.020	0.08	3.3
	0.101	0.41	0.8
	0.501	2.03	2.7
	2.51	10.2	2.2
	10.0	40.4	3.4
	49.9	202	2.0
	200	808	0.0
Monkey	0.020	0.08	0.0
	0.101	0.41	0.0
	0.501	2.03	0.0
	2.51	10.2	0.0
	10.0	40.4	0.0
	49.9	202	2.0
	200	808	0.0

Report Title	Study Type	Test Article	Report Number
Protein Binding of 524W91 in Human, Monkey, Mouse, and Rabbit Plasma	Plasma Protein Binding	FTC	TBZZ/93/0025

Species	FTC concentration		% Bound
	µg/mL	µM	
Mouse	0.020	0.08	0.7
	0.101	0.41	3.6
	0.501	2.03	0.0
	2.51	10.2	2.3
	10.0	40.4	0.0
	49.9	202	3.0
	200	808	3.4
Rabbit	0.020	0.08	2.3
	0.101	0.41	0.0
	0.501	2.03	1.3
	2.51	10.2	0.0
	10.0	40.4	0.0
	49.9	202	0.0
	200	808	0.4

524W91 = emtricitabine

6.2. RPV

6.2.1. TMC278–NC112: The Plasma Protein Binding and Blood Distribution of RPV in Animals and Man

Report Title	Study Type	Test Article	Report Number
The Plasma Protein Binding and Blood Distribution of TMC278 in Animals and Man	Plasma Protein Binding	[^{3}H]RPV	TMC278-NC112

Method:

The plasma protein binding of TMC278 was studied by equilibrium dialysis of plasma samples after fortification with ^{3}H-labeled TMC278. Plasma was subjected to equilibrium dialysis against a 0.067 M phosphate buffer, pH 7.17, at 37°C for 3 hours. Concentration of ^{3}H –TMC278 in dialysis compartments was determined by liquid scintillation counting. The binding of TMC278 to purified human serum albumin and α1-acid glycoprotein was also investigated by equilibrium dialysis. In blood distribution studies, samples of whole blood were combusted in an oxidizer and ^3H_2O captured and counted by liquid scintillation.

Species	Swiss CD-1 Mouse[a] (Male)		Swiss CD-1 Mouse[a] (Female)		Sprague Dawley Rat[a] (Male)		Sprague Dawley Rat[a] (Female)		New Zealand White Rabbit[b] (Female)		Beagle Dog[b] (Male)		Human[b] (Male)	
Parameters Measured														
Concentration Tested in Plasma (µg/mL)	0.01-100		0.01-100		0.01-100		0.01-100		0.01-100		0.01-100		0.01-3	
Plasma Bound % at 1 µg/mL[c]	99.93		99.94		99.84		99.86		99.97		99.35		99.67	
Concentration Tested in Blood (µg/mL)	0.1	1	0.1	1	0.1	1	0.1	1	0.1	1	0.1	1	0.1	1
Blood-to-Plasma Ratio	0.60	0.60	0.58	0.58	0.69	0.67	0.67	0.67	0.61	0.61	0.69	0.68	0.67	0.66
Distribution to: Plasma Water (%)	0.12	0.07	0.06	0.07	0.16	0.15	0.13	0.14	0.02	0.03	0.5	0.5	0.3	0.2
Plasma Proteins (%)	101	100	102	103	90.5	93.0	94.1	94.1	102	103	72.6	73.4	77.3	78.1
Blood Cells (%)	-0.9	-0.2	-1.7	-3.0	9.3	6.8	5.8	5.8	-1.8	-2.8	26.9	26.2	22.4	21.7
Hematocrit (%)	40		41		38		38		38		51		48	

Additional Information

Preliminary studies evaluated: (a) length of time needed to reach equilibrium in the dialysis cells (3 hours was deemed sufficient); (b) the effect of pH (as pH increased from 5.1 to 8.4, the percentage bound increased from 99.59 to 99.80, so pH was standardized at 7.4 for human plasma); and (c) binding to purified human plasma protein (at physiological concentrations, TMC278 (0.01 to 3 µg/mL) bound to serum albumin (99.50%) and α_1-acid glycoprotein (between 25.9% and 55.0%).

a for rat and mouse, each value represents a mean of 4 observations (2 pools x 2);
b for rabbit, dog and man, each value represents a mean of 10 observations (5 individual samples x 2);
c Over the concentration range tested the fraction bound to plasma remained nearly the same

6.2.2. TMC278–NC332: The Distribution of ^{14}C-RPV in Blood and Protein Binding of ^{14}C-RPV in Plasma from Monkey and Guinea Pig

Report Title	Study Type	Test Article	Report Number
The Distribution of ^{14}C-TMC278 in Blood and Protein Binding of ^{14}C-TMC278 in Plasma from Monkey and Guinea Pig	Plasma Protein Binding	[^{14}C]RPV	TMC278-NC332

Method:
The plasma protein binding of TMC278 was studied by equilibrium dialysis of plasma samples after fortification with ^{14}C-labeled TMC278. Plasma was subjected to equilibrium dialysis against a 0.067 M phosphate buffer, pH 7.17, at 37°C for 4 hours. Concentration of ^{14}C –TMC278 in dialysis compartments was determined by liquid scintillation counting. In blood distribution studies, samples of whole blood were combusted in an oxidizer and $^{14}CO_2$ captured and counted by liquid scintillation.

Species	Dunkin Hartley Guinea Pig		Cynomolgus Monkey	
Parameters Measured				
Concentration Tested in Plasma (µg/mL)	2.5	8	2.5	5
Plasma Bound % at 1 µg/mL [c]	99.87	99.87	99.14	99.08
Concentration Tested in Blood (µg/mL)	2.5	8	2.5	5
Blood-to-Plasma Ratio	0.64	0.63	0.96	0.94
Distribution to: Plasma Water (%)	0.11	0.11	0.53	0.59
Plasma Proteins (%)	84.71	85.23	61.84	63.46
Blood Cells (%)	15.18	14.66	37.63	35.96
Hematocrit (%)	46		40	

Additional Information

6.3. TAF and TFV

6.3.1. AD-120-2026: Plasma Protein Binding of TAF In Vitro

Report Title	Study Type	Test Article	Report Number
Plasma Protein Binding of GS-7340	Plasma protein binding, in vitro	TAF	AD-120-2026

Study System: Plasma from Dog and Human

Target Entry, Test System, and Methods: Equilibrium dialysis for 3 hours at 37°C against 0.133 M phosphate buffer, pH 7.4. Analysis by LC/MS/MS.

Matrix	Fraction Unbound (%)
Dog plasma	48.0 ± 6.2
Human plasma	46.8 ± 2.3

TAF = tenofovir alafenamide
Note: Incubation concentration of TAF was 2 μM

6.3.2. P0504-00039.1: Protein Binding of TFV

Report Title	Study Type	Test Article	Report Number
Protein Binding of Cidofovir, Cyclic HPMPC, PMEA, and PMPA in Human Plasma and Serum	Plasma Protein Binding, In Vitro	TFV	P0504-00039.1

Study System	In vitro
Target Entity, Test System & Method	Plasma/ serum, ultrafiltration

Matrix	TFV Concentration	% Unbound
Human Plasma	0.01 µg/mL	96.9
	2.01 µg/mL	99.9
	5.01 µg/mL	101.0
	10.01 µg/mL	95.0
	25.01 µg/mL	103.5
		99.3 (3.3) Mean (SD)
Human Serum	0.01 µg/mL	90.6
	2.01 µg/mL	92.4
	5.01 µg/mL	97.7
	10.01 µg/mL	88.5
	25.01 µg/mL	94.7
		92.8 (3.6) Mean (SD)

HPMPC = cidofovir; PMEA = adefovir; PMPA = tenofovir

7. PHARMACOKINETICS: STUDY IN PREGNANT OR NURSING ANIMALS

7.1. FTC

7.1.1. TOX103: Toxicokinetic Study to Determine Fetal Exposure of FTC in Mice

Report Title	Study Type	Test Article	Report Number
Toxicokinetic Study to Determine Fetal Exposures in CD-1 Mice Given TP-0006 Orally	Repeat-Dose Tissue Distribution	FTC	TOX103 Report Addendum

Methods:

Species	Mouse / CD-1
Gestation Day	Gestation days 6-15
Vehicle/Formulation	0.5% aqueous methylcellulose
Method of Administration	Oral gavage
Dose (mg/kg)	1000 (plus 500 only on GD 15)
Analyte	FTC
Assay	LC-MS (SIM)

Plasma:

The mean ± standard deviation for plasma concentrations:

Pregnant mice	137.1 ± 28.0 µg/mL
Pooled fetal homogenate	57.7 ± 10.4 µg/mL

The mean fetal/maternal concentration ratio was 0.41 ± 0.04 µg/mL

Additional Information: One female in the low dose group was not pregnant and was not included in the mean values.

7.1.2. TOX038: Effects of FTC on Embryo/Fetal Development in Rabbits - Toxicokinetics

Report Title	Study Type	Test Article	Report Number
A Study of the Effects of TP-0006 on Embryo/Fetal Development in Rabbits	Repeat-Dose Tissue Toxicokinetics in Embryo/Fetus	FTC	TOX038 Report Addendum

Methods:

Species	Rabbit / New Zealand White
Gestation Day/Number of Animals	Treated on gestation day 7-19 / 20 females per group
Vehicle/Formulation	0.5% aqueous methylcellulose
Method of Administration	Oral
Dose (mg/kg)	0, 100, 300, 1000
Analyte	FTC
Assay	LC/MS/MS

Toxicokinetics

Emtricitabine was rapidly absorbed with C_{max} occurring generally within 1 hour postdose. AUC and C_{max} increased linearly with dose. Plasma elimination t½ was 3–4 hours at all dose levels. Fetal/maternal exposure ratios were around 0.4–0.5 one hour after dosing (at t_{max}) for all dose levels. Emtricitabine is readily transferred across the placenta.

Dose (mg/kg/day)	C_{max} (µg/mL)	t_{max} (h)	AUC_{0-12} (µg•h/mL)	AUC_{0-24} (µg•h/mL)	Fetal/Maternal Ratio
100	16.0	1.0	43.6	87.3	0.42
300	44.2	1.4	157.6	315.2	0.51
1000	143.3	1.7	628.9	1257.8	0.41

Additional Information: A NOEL of 100 mg/kg/day was established for maternal toxicity.

7.2. RPV

7.2.1. TMC278-NC105: Oral Developmental Toxicity Study in the Rat

Report Title			**Study Type**		**Test Article**	**Report Number**
Oral Developmental Toxicity Study in the Rat			Repeat-Dose Tissue Distribution		RPV	TMC278-NC105
Species	Rat (pregnant Sprague Dawley)					
Feeding Condition	Not fasted					
Vehicle/Formulation	TMC278 base in PEG400/CA (10%)					
Route	Oral (gavage)					
Gender (M/F)/Number of Animals	F/6	F/4	F/6	F/4	F/6	
Dose (mg/kg/day)	40		120		400	
Concentration (mg/mL)	4		12		40	
Duration of Dosing (day)	1 (GD6)	11 (GD16)	1 (GD6)	11 (GD16)	1 (GD6)	11 (GD16)
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma	
Analyte	TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters						
C_{max} (µg/mL)	4.9	5.6	6.0	7.2	14	13
t_{max} (h)	1.0	2.0	0.5	1.0	8.0	8.0
AUC (µg.h/mL)	33	37	65	63	182	152
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	3.3	3.8	4.2	4.0	5.5	5.2
(Time for calculation –h)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)

Additional Information
The sampling times were 0.5, 1, 2, 4, 8 and 24 h after dose administration
CA: citric acid; GD: gestation day; LC-MS/MS: liquid chromatography with tandem mass spectrometry; PEG400: polyethylene glycol 400

7.2.2. TMC278–NC128: Pilot Oral Developmental Toxicity Study in the Rabbit

Report Title	**Study Type**	**Test Article**	**Report Number**
Pilot Oral Developmental Toxicity Study in the Rabbit	Repeat-Dose Tissue Distribution	RPV	TMC278-NC128 (pilot study)

Species	Rabbit (pregnant New Zealand white)		
Feeding Condition	-		
Vehicle/Formulation	TMC278 base in aqueous HPMC (0.5%)		
Route	Oral (gavage)		
Gender (M/F)/Number of Animals	F/3	F/3	F/3
Dose (mg/kg/day)	25	75	150
Concentration (mg/mL)	5	15	30
Duration of Dosing[a] (day)	12 (GD17)	12 (GD17)	12 (GD17)
Sample (whole blood, plasma, serum, etc.)	plasma	plasma	plasma
Analyte	TMC278	TMC278	TMC278
Assay	NS	NS	NS
Pharmacokinetic Parameters			
C_{max} (µg/mL)	18	39	63
t_{max} (h)	6.7	6.7	13
AUC (µg.h/mL)	327	685	1252
(Time for calculation –h)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	ND	ND	ND
(Time for calculation –h)	-	-	-

Additional Information
The sampling times were 0 (predose), 1, 2, 4, 8 and 24 h on Day 12 of dosing (GD17)
a pregnant animals were dosed once daily from Day 6 to Day 19 inclusive
GD: gestation day; HPMC: hydroxypropyl-methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not determined; NS: not specified.

7.2.3. TMC278–NC130: Oral Developmental Toxicity Study in the Rabbit

Report Title			**Study Type**		**Test Article**	**Report Number**	
Pilot Oral Developmental Toxicity Study in the Rabbit			Repeat-Dose Tissue Distribution		RPV	TMC278-NC130 (main study)	
Species	Rabbit (pregnant New Zealand white)						
Feeding Condition	Not fasted						
Vehicle/Formulation	TMC278 base in aqueous HPMC (0.5%)						
Route	Oral (gavage)						
Gender (M/F)/Number of Animals	F/3		F/3		F/3		
Dose (mg/kg/day)	5		10		20		
Concentration (mg/mL)	1		2		4		
Duration of Dosing[a] (day)	1 (GD 6)	14 (GD 19)	1 (GD 6)	14 (GD 19)	1 (GD 6)	14 (GD 19)	
Sample (whole blood, plasma, serum, etc.)	plasma	plasma	plasma	plasma	plasma	plasma	
Analyte	TMC278	TMC278	TMC278	TMC278	TMC278	TMC278	
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	
Pharmacokinetic Parameters							
C_{max} (µg/mL)	6.4	6.7	9.7	10	13	15	
t_{max} (h)	11	11	11	8.0	9.3	11	
AUC (µg.h/mL)	95	105	162	170	219	232	
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	
$t_{1/2}$ (h)	8.2	7.6	12	8.4	11	8.6	
(Time for calculation –h)	-	-	-	-	-	-	

Additional Information
The sampling times were 0 (predose), 1, 2, 4, 8, 12 and 24 h after dosing.
a pregnant animals were dosed once daily from Day 6 to Day 19 of gestation inclusive.
GD: gestation day; HPMC: hydroxypropyl-methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry

7.3. TAF and TFV

7.3.1. TAF

Studies of TAF in pregnant animals are presented in m2.6.7, Sections 11.4 and 12.6.

7.3.2. TFV

7.3.2.1. 96-DDM-1278-005: Placental Transfer and Pharmacokinetics of TFV in Infant Rhesus Monkeys

Report Title	Study Type	Test Article	Report Number
Placental Transfer and Pharmacokinetics of PMPA (GS-1278) in Infant Rhesus Monkeys	Placental Transfer	TFV	96-DDM-1278-005

Methods:

Species	Monkey
Gestation Day/ Number of Animals	Daily dosing beginning at gestational day 111, one animal
Vehicle Formulation	Aqueous suspension
Method of Administration	SC
Dose (mg/kg)/ day	30
Analyte	TFV
Assay	HPLC

Serum Concentration TFV (µg/mL) 30 mins After Administration

Time (gestational day)	115	127	134	140	151	Mean (SD) [CV%]
Fetal	7.9	9.1	10.1	15	5.9	9.6 (3.4) [35.4]
Maternal	45.6	61.2	69.4	53.7	56.3	57.2 (8.84) [15.4]
Fetal/Maternal Ratio	0.17	0.15	0.15	0.28	0.11	0.17 (0.07) [38.6]

Additional Information: Based upon the data above it was concluded that placental transfer of TFV appeared to be significant.

7.3.2.2. P2000116: Pharmacokinetics of TFV in Lactating Rhesus Monkeys

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics of Tenofovir in Healthy Adult Female Lactating Rhesus Monkeys Following a Single 30 mg/kg Subcutaneous Dose of Tenofovir	Lactating Animals	TFV	P2000116

Methods:

Species	Monkey
Gestation Day/ Number of Animals	Healthy adult female lactating animals
Method of Administration	SC
Dose (mg/kg)/ day	30 single dose
Analyte	TFV
Assay	LC/MS/MS

	Animal 1		Animal 2	
PK Parameters (n=2)	**Milk**	**Serum**	**Milk**	**Serum**
C_{max} (µg/mL)	0.808	18.3	0.610	30.2
t_{max} (h)	4	0.5	1	0.5
$AUC_{0-\infty}$ (µg•h/mL)	12.8	68.9	12.1	56.2
AUC Extrapolated (%)	21.7	0.219	23.3	0.193
C_{last} (µg/mL)	0.188	0.0264	0.179	0.0264
t_{last} (h)	24	24	24	24
CL/F (mL/h/kg)	2338	435	2482	534
$MRT_{0-\infty}$ (h)	16.1	2.79	17.0	3.14
$t½\lambda_z$ (h)	10.3	3.97	10.9	2.85
V_z/F (mL/kg)	34740	2489	39133	2191

8. PHARMACOKINETICS: METABOLISM IN VITRO

8.1. FTC

8.1.1. 15396v1: Human Cytochrome P450 Reaction Phenotyping and Glucuronidation Potential of FTC

Report Title	Study Type	Test Article	Report Number
Identification of the Principal Human Cytochrome P450 Isoenzyme(s) and Potential Glucuronidation Responsible for the Metabolism of Emtricitabine (FTC) using Pooled Human Liver Microsomes and Bactosomes Containing cDNA-expressed Human Cytochrome P450 (CYP) Isoenzymes	Metabolism	FTC	15396V1

Study System	Study system 1) cDNA-Expressed Human Cytochromes P450 (CYP1A2, 2A6, 2B6, 2C8, 2C9 2C19, 2D6, 2E1 and 3A4) Study system 2) Pooled Human Hepatic Microsomal Fraction and Enzyme-Selective Inhibitors
Results	• One minor metabolite (~1%) was detected in cDNA-expressed CYP3A4 incubations • No metabolites were formed by CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6 or 2E1 • Microsomal incubations in the presence and absence of selective inhibitors confirmed the low rate of metabolism, and also suggested the possible involvement of flavin-containing monooxygenase enzymes (due to lack of complete inhibition by the CYP3A-selective inhibitor, ketoconazole • No glucuronidation was observed pooled human liver microsomal fraction in the presence of UDPGA
Conclusions	• Emtricitabine was relatively stable in the presence of cytochrome P450 enzymes and is not a substrate for hepatic glucuronidation

8.2. RPV

8.2.1. TMC278-NC102: Metabolism of RPV In Vitro (Hepatocytes and Liver Subcellular Fractions)

Report Title	Study Type	Test Article	Report Number
The In-Vitro Metabolism of ^{14}C-TMC278 in Hepatocytes and Liver Subcellular Fractions of Male and Female Swiss Albino Mice, Male and Female Black Agouti rasH2 Microinjected Mice, Male and Female Rats, Female Rabbit, Male Dog and Man	Metabolism, In Vitro	[^{14}C]RPV	TMC278-NC102

Method:
^{14}C-TMC278 (5 µM) was incubated for various time periods with hepatocytes (suspensions and primary cultures) and with liver subcellular fractions (microsomes and 12,000 x g supernatant fractions). Samples were analyzed by radio-HPLC. Co-chromatography, enzyme [redacted], LC-MS/MS and NMR techniques were used for identification of the metabolites.

	Percentage of injected sample radioactivity for unchanged compound and its metabolites															
Study System	**Mouse (male, Swiss albino)**				**Mouse (female, Swiss albino)**				**Mouse (male, black Agouti rasH2)**				**Mouse (female, black Agouti rasH2)**			
Metabolites	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**
Parent (UD)	11.8	3.9	57.1	85.3	3.2	4.4	71.9	81.6	13.1	3.9	49.1	72.4	6.1	1.9	57.7	77.3
2	-	0.5	-	0.6	-	0.7	-	1.0	-	-	-	0.9	-	-	0.7	0.6
3	0.6	1.0	-	-	1.0	0.6	-	-	-	-	-	-	-	-	-	-
4+5	4.1	2.0	-	0.5	7.2	5.2	-	-	6.6	4.6	0.7	-	8.6	4.6	0.6	-
6	4.8	2.9	0.7	-	3.5	2.2	-	0.7	8.2	3.1	0.8	-	4.1	3.6	0.7	-
7	2.8	1.4	1.1	-	1.0	-	0.6	-	-	-	-	-	-	-	-	-
8	1.8	2.9	1.4	-	3.3	6.7	0.8	-	3.0	3.5	2.3	-	4.5	5.3	4.0	-
10	61.9	49.7	35.9	-	7.6	12.6	17.1	-	-	-	-	-	-	-	-	-
10 (+12[*] or +14[**])									28.3	43.9	36.0[*]	-	15.0[**]	36.4	23.8[*]	-
13	-	-	-	0.9	-	-	-	1.3	-	-	3.3	0.8	-	-	0.7	0.5
14	-	18.4	-	-	6.0	9.2	-	-	5.3	18.0	-	-	-	15.3	-	-
17	1.5	5.3	-	0.7	3.2	8.9	-	-	5.2	7.9	-	0.9	5.7	6.7	-	0.6
18	-	10.5	-	-	3.0	0.6	-	-	-	11.1	-	-	-	5.2	-	-
22	-	-	0.9	2.4	-	-	0.6	3.9	-	-	-	5.5	-	-	-	3.2

Report Title	Study Type	Test Article	Report Number
The In-Vitro Metabolism of ^{14}C-TMC278 in Hepatocytes and Liver Subcellular Fractions of Male and Female Swiss Albino Mice, Male and Female Black Agouti rasH2 Microinjected Mice, Male and Female Rats, Female Rabbit, Male Dog and Man	Metabolism, In Vitro	[^{14}C]RPV	TMC278-NC102

Method:

^{14}C-TMC278 (5 µM) was incubated for various time periods with hepatocytes (suspensions and primary cultures) and with liver subcellular fractions (microsomes and 12,000 x g supernatant fractions). Samples were analyzed by radio-HPLC. Co-chromatography, enzyme [redacted], LC-MS/MS and NMR techniques were used for identification of the metabolites.

	Percentage of injected sample radioactivity for unchanged compound and its metabolites															
Study System	**Mouse (male, Swiss albino)**				**Mouse (female, Swiss albino)**				**Mouse (male, black Agouti rasH2)**				**Mouse (female, black Agouti rasH2)**			
Metabolites	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**
25	13.3	7.3	-	-	49.3	46.9	-	-	25.5	6.5	-	-	45.2	23.7	-	-
27	1.8	-	0.9	1.1	-	-	1.1	2.5	2.2	1.0	0.8	2.4	1.2	-	1.3	2.0
33	0.8	-	0.9	2.2	1.8	-	1.2	2.8	2.4	-	0.8	2.6	3.5	-	1.5	1.9
35+36	0.9	-	0.7	2.6	1.8	-	1.2	4.1	1.4	-	0.5	1.9	1.8	-	2.3	2.9
38	-	-	-	-	0.9	0.7	0.8	0.6	-	-	0.6	-	-	-	1.3	-
42	-	0.5	1.3	1.4	0.9	1.6	5.4	2.2	-	0.7	2.9	2.1	0.9	0.7	9.6	1.5
43	0.9	1.5	0.8	0.5	-	-	1.6	0.4	0.7	0.7	1.9	0.9	0.4	-	2.7	0.4
Sum	**107.0**	**107.8**	**101.7**	**98.2**	**93.7**	**100.3**	**102.3**	**101.1**	**101.9**	**104.9**	**99.7**	**90.4**	**97.0**	**103.4**	**106.9**	**90.9**

- : not detected; 12,000g: 12,000 x g supernatant fractions; HPLC: high performance liquid chromatography; LC-MS/MS: liquid chromatography with tandem mass spectrometry; MICR: microsomes; NMR: nuclear magnetic resonance; PCK: primary culture; SK: suspension culture; UD: unchanged drug

* The figures represent the sum of the % of M10 and M12; ** The figures represent the sum of the % of M10 and M14

Report Title	Study Type	Test Article	Report Number
The In-Vitro Metabolism of ^{14}C-TMC278 in Hepatocytes and Liver Subcellular Fractions of Male and Female Swiss Albino Mice, Male and Female Black Agouti rasH2 Microinjected Mice, Male and Female Rats, Female Rabbit, Male Dog and Man	Metabolism, In Vitro	[^{14}C]RPV	TMC278-NC102

	Percentage of injected sample radioactivity for unchanged compound and its metabolites																			
Study System	**Rat (male, Sprague-Dawley)**				**Rat (female, Sprague-Dawley)**				**Rabbit (female, New Zealand)**				**Dog (male, Beagle)**				**Man**			
Metabolites	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**
Parent (UD)	59.5	8.2	42.2	90.2	47.7	18.2	64.0	96.4	20.2	1.0	28.7	87.4	72.3	25.4	91.5	90.7	75.8	23.1	34.8	43.5
2	-	-	-	-	-	-	-	-	1.3	-	1.3	-	-	0.9	-	-	-	-	4.8	1.6
3	-	-	-	-	-	-	-	-	0.8	-	1.1	-	-	0.5	-	-	0.2	1.5	-	-
4	0.6	2.1	-	-	0.3	1.4		-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	5.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	0.5	1.0	3.5	-	-	0.5	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	1.5	0.7	3.9	-	0.7	2.0	-	-	0.4	1.8	2.3	1.3
8	0.5	1.6	4.3	-	0.7	2.1	1.5	-				-	-	-	-	-	-	-	-	-
10	35.6	56.4	40.7	-	46.7	58.7	25.7	-	3.8	3.0	2.1	1.0	1.2	6.4	1.4	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.2	4.8	-	-
14	-	5.6	-	-	-	-	-	-	-	-	-	-	1.5	4.2	-	-	1.5	4.9	-	-
15	-	-	-	-	-	-	-	-	15.8	11.3	-	-	-	-	-	-	1.1	4.6	-	-
17	0.8	7.1	0.6	1.1	3.2	22.5	-	-	5.3	8.8	-	-	-	-	-	-	0.5	6.8	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.7	-	-
19	-	-	-	-	-	-	-	-	7.6	11.9	4.8	1.3	3.7	9.4	-	-	0.2	2.1	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.5	0.9	-	-	-	-
22	-	-	-	-	-	-	0.4	0.7	-	-	-	-	-	-	-	-	-	-	3.6	7.8
25	0.8	1.1	-	-	1.7	3.5	-	-	38.8	46.2	5.4	-	8.6	22.0	-	-	11.8	31.0	-	-
26	-	-	-	-	-	-	-	-	-	-	-	-	2.6	6.7	-	-	-	-	-	-
27	0.4	0.7	0.9	0.7	0.6	-	-	-	-	-	11.8	0.4	-	-	2.0	0.8	2.4	7.2	5.6	4.9

Report Title	Study Type	Test Article	Report Number
The In-Vitro Metabolism of ^{14}C-TMC278 in Hepatocytes and Liver Subcellular Fractions of Male and Female Swiss Albino Mice, Male and Female Black Agouti rasH2 Microinjected Mice, Male and Female Rats, Female Rabbit, Male Dog and Man	Metabolism, In Vitro	[^{14}C]RPV	TMC278-NC102

	Percentage of injected sample radioactivity for unchanged compound and its metabolites																			
Study System	**Rat (male, Sprague-Dawley)**				**Rat (female, Sprague-Dawley)**				**Rabbit (female, New Zealand)**				**Dog (male, Beagle)**				**Man**			
Metabolites	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**
30+31	-	-	-	-	-	-	-	-	-	-	4.6	1.2	-	-	0.5 + 0.8	-	0.9*	1.6*	3.6	0.5*
32	-	1.3	-	-	-	6.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	-	-	1.0	1.1	-	-	-	-	-	-	4.6	1.2	-	-	2.3	1.0	1.4	1.1	2.5	2.9
34	-	-	-	-	-	-	-	-	-	-	-	-	1.7	6.0	-	-	-	-	-	-
35+36	-	-	-	-	-	-	-	-	-	-	-	-	-	1.3	0.5	1.2	0.4		9.8	7.9
36	-	-	1.1	2.1	-	-	0.5	1.2	2.5		6.0	1.4	-	-	-	-	-	-	-	-
38	-	-	0.7	2.0	-	-	-	0.5	-	-	6.9	0.4	-	-	0.6	-	-	-	3.8	-
42	-	-	1.1	2.0	-	-	1.2	1.1	-	-	5.1	0.8	1.7	2.6	3.5	1.2	0.5	1.0	6.6	6.0
43	2.8	-	3.8	0.9	2.0	0.8	3.8	1.4	-	-	1.2	1.4	-	0.6	0.9	0.9	1.8	0.6	Trace	-
													-	-	-	-	-	-	-	-
Sum	**101.5**	**85.1**	**105.3**	**101.4**	**102.9**	**113.8**	**97.7**	**101.3**	**97.6**	**82.9**	**87.5**	**96.5**	**94.0**	**88.0**	**105.0**	**96.7**	**100.9**	**93.6**	**73.8**	**76.4**

- : not detected; 12,000g: 12,000 x g supernatant fractions; HPLC: high performance liquid chromatography; LC-MS/MS: liquid chromatography with tandem mass spectrometry; MICR: microsomes; NMR: nuclear magnetic resonance; PCK: primary culture; SK: suspension culture; UD: unchanged drug.
* Figure represents only M30

8.2.2. TMC278-NC333: Metabolism of RPV In Vitro (Liver 12,000 x g Supaernatant and Hepatocytes)

Report Title	Study Type	Test Article	Report Number
The In Vitro Metabolism of ^{14}C-TMC-278 in Liver 12000 x g Supernatant and Hepatocytes of Guinea Pig and Monkey	Metabolism, In Vitro	[^{14}C]RPV	TMC278-NC333

Method:
^{14}C-TMC278 (5 µM) was incubated with hepatocytes (primary cultures) for approximately 24 hours and with liver subcellular fractions (12,000 x g supernatant fractions) for 120 minutes. Samples were analyzed by radio-HPLC and metabolites identified by LC-MS/MS.

	Percentage of injected sample radioactivity for unchanged compound and its metabolites			
Study System	**Guinea pig (female, Dunking Hartley)**		**Monkey (male, Cynomolgus)**	**Monkey (female, Cynomolgus)**
Metabolites	**PCK**	**12,000g**	**PCK**	**12,000g**
Parent (UD)	2.5	30.9	9.1	12.6
M10	ND	55.0	ND	ND
M12	45.8	ND	10.7	ND
M13+M14	19.1	ND	ND	ND
M17+M18	11.1	ND	ND	ND
M19	ND	ND	7.4	ND
M22	ND	ND	ND	8.3
M25	7.1	ND	53.5	ND
M27	ND	ND	ND	10.6
M30	2.6	2.8	ND	ND
M33	ND	ND	ND	12.7
M38	ND	ND	ND	9.2
M42	ND	ND	ND	9.4
Sum	**88.2**	**88.7**	**80.6**	**62.7**

12,000g: 12,000 x g supernatant fractions; HPLC: high performance liquid chromatography; LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not detected; PCK: primary culture; UD: unchanged drug

8.2.3. TMC278-NC141: Metabolism of RPV In Vitro (CYP Reaction Phenotyping and Kinetics)

Report Title	Study Type	Test Article	Report Number
An In Vitro Study to (a) Identify the Microsomal Cytochrome P-450 Iso-enzymes Mediating TMC278 Metabolism (Reaction Phenotyping) and to (b) Determine the Kinetics of TMC278 Metabolism in Human Liver Microsomes	Metabolism, In Vitro	[^{14}C]RPV	TMC278-NC141

Method:
^{14}C-TMC278 was incubated at various concentrations in human liver microsomes for 15 minutes at a protein concentration of 0.25 mg/ml. The amount of unchanged TMC278 remained in the samples was determined by radio-HPLC and the % metabolized (substrate turnover rate) was calculated. The kinetic parameters were calculated by a Michaelis-Menten equation using validated Winnonlin software (Pharsight, Winnonlin 4.0.1).

	I. Protein concentration: 0.25 mg/ml
Substrate Conc. (µM)	**Product rate (pmol/mg/min)[a]**
0.5	32.2, 36.8, 28.5
1	60.7, 57.2, 56.2
3	146, 124, 150
5	206, 222, 248
7.5	281, 199, 205
10	327, 420, 223
15	287, 299, 231
20	270, 308, 350
30	317, 341, 429
50	356, 396, 222
K_m (± std error)	**4.17 (± 1.06) µM**
V_{max} (± std error)	**381 (± 26) pmol/min/mg protein**

Additional information
a Triplicate values are used in the determination of K_m and V_{max}

Report Title	Study Type	Test Article	Report Number
An In Vitro Study to (a) Identify the Microsomal Cytochrome P-450 Iso-enzymes Mediating TMC278 Metabolism (Reaction Phenotyping) and to (b) Determine the Kinetics of TMC278 Metabolism in Human Liver Microsomes	Metabolism, In Vitro	[^{14}C]RPV	TMC278-NC333

Method:

Inhibition of the metabolism of TMC278 in human liver microsomes by diagnostic inhibitors was carried out with ^{14}C-TMC278 (5 µM) for 15 minutes at a protein concentration of 0.25 mg/ml. The amounts of unchanged TMC278 and its metabolites (M27, M33, M35+M36[a] and M42[a]) were determined by radio-HPLC. The values represent the percentage of inhibition obtained for each inhibitor in comparison to a control incubate (without inhibitor). Each value represents mean of three observations.

		% Inhibition of Metabolism[b]				
Diagnostic Inhibitor	**CYP P450 Form**	**Overall[c]**	**M27[c]**	**M33[c]**	**M35+M36[a]**	**M42[a,b]**
Furafylline (10 µM)	CYP1A2	-10.9	-10.9	-13.9	66.7	-22.3
Coumarin (100 µM)	CYP2A6	-9.3	-25.0	-44.4	75.0	-13.4
Sulphaphenazole (10 µM)	CYP2C8/9/10	-3.0	0.0	30.6	86.1	-25.1
Quinidine (10 µM)	CYP2D6	4.4	1.56	-5.56	69.4	-3.24
4-methylpyrazole (20 µM)	CYP2E1	-6.5	1.56	-8.33	63.9	-21.1
Ticlopidine (5 µM)	CYP2C19/D6	-3.1	-2.94	-27.8	25.0	-7.91
Ketoconazole (1 µM)	CYP3A4	107	100	100	100	100
Troleandomycin (200 µM)	CYP3A4	107	100	100	100	100
Clarithromycin (15 µM)	CYP3A	57.2	45.3	38.9	58.3	57.1
Ritonavir (0.15 µM)	CYP3A	91.3	100	100	100	80.2
1-aminobenzotriazole	CYP P450	104	100	100	100	100

Additional Information

a Major metabolite in human liver microsomes (> 5 % of the sample radioactivity)

b Calculated from control incubation (without inhibitor); higher the positive value and higher the extent of inhibition

c Negative values indicates higher % product formation in test sample compared to the control. This was more prominent with the minor metabolites. For all qualitative purposes, all negative values were considered as no inhibition.

Report Title	Study Type	Test Article	Report Number
An In Vitro Study to (a) Identify the Microsomal Cytochrome P-450 Iso-enzymes Mediating TMC278 Metabolism (Reaction Phenotyping) and to (b) Determine the Kinetics of TMC278 Metabolism in Human Liver Microsomes	Metabolism, In Vitro	[^{14}C]RPV	TMC278-NC333

Method:

the metabolism of TMC278 in *E. coli* expressed CYP systems (prepared in-house) was carried out with ^{14}C-TMC278 (5 µM) for 60 minutes at a CYP P450 concentration of 100 pmol/ml of incubation. The amounts of unchanged TMC278 and its metabolites (M50, M2, M22[a], M27, M33[a], M35+M36[a], M51, and M42[a]) were determined by radio-HPLC. Each value represents mean ± S.D of three observations.

Cytochrome P-450 Form	Overall	Product formation rate (pmol/min. 100 pmol P450)							
(100 pmol/ml)	% Metabolism[b]	M50	M2	M22	M27	M33	M35+M36	M51	M42
CYP1A2	1.40 ± 0.26	-	-	-	-	-	-	-	-
CYP2A6	1.07 ± 1.44	-	-	-	-	-	-	-	-
CYP2B6	1.30 ± 1.76	-	-	-	-	-	-	-	-
CYP2C8	0.43 ± 0.38	-	-	-	-	-	-	-	-
CYP2C9	0.47 ± 0.42	-	-	-	-	-	-	-	-
CYP2C19	0.37 ± 0.32	-	-	-	-	-	-	-	-
CYP2D6	1.07 ± 1.85	-	-	-	-	-	-	-	-
CYP2E1	0.00 ± 0.00	-	-	-	-	-	-	-	-
CYP3A4	86.87 ± 1.40	4.11 ± 0.35	9.03 ±0.71	15.6 ± 1.30	2.58 ± 0.95	3.00 ± 0.58	20.4 ± 2.95	-	6.28 ± 0.75
CYP3A5	0.53 ± 0.68	-	-	-	-	-	-	-	0.31 ± 0.34

Additional Information

a Major metabolites in human liver microsomes (> 5 % of the sample radioactivity)
b Overall % metabolism of TMC278 calculated from % drug that remained in the sample at the end of the incubation
- No measurable product observed in radio-HPLC profile (LLOQ = 211 dpm)

Report Title	Study Type	Test Article	Report Number
An In Vitro Study to (a) Identify the Microsomal Cytochrome P-450 Iso-enzymes Mediating TMC278 Metabolism (Reaction Phenotyping) and to (b) Determine the Kinetics of TMC278 Metabolism in Human Liver Microsomes	Metabolism, In Vitro	[^{14}C]RPV	TMC278-NC333

Method:

The metabolism of TMC278 in expressed CYP systems (Supersomes®) was carried out with ^{14}C-TMC278 (5 µM) for 60 minutes at a CYP P450 concentration of 100 pmol/ml of incubation. The amounts of unchanged TMC278 and its metabolites (M52, M2, M22[a], M27, M33 [a], M35+M36[a], M51[a] and M42[a]) were determined by radio-HPLC. Each value represents mean ± S.D of three observations.

Cytochrome P-450 Form	Overall	Product formation rate (pmol/min. 100 pmol P450)							
(100 pmol/ml)	% Metabolism[b]	M52	M2	M22	M27	M33	M35+M36	M51	M42
CYP1A2	± 0.64	-	-	-	-	-	-	-	-
CYP2A6	0.37 ± 0.32	-	-	-	-	-	-	-	-
CYP2B6	0.50 ± 0.44	-	-	-	-	-	-	-	-
CYP2C8	0.00 ± 0.00	-	-	-	-	-	-	-	-
CYP2C9	0.43 ± 0.38	-	-	-	-	-	-	-	-
CYP2C19	0.43 ± 0.40	-	-	-	-	-	-	-	-
CYP2D6	2.37 ± 2.45	-	-	-	-	-	-	-	-
CYP2E1	0.23 ± 0.40	-	-	-	-	-	-	-	-
CYP3A4	39.5 ± 2.1	0.97 ± 0.84	4.44 ± 0.86	3.39 ± 0.32	4.14 ± 0.68	4.00 ± 0.46	7.72 ± 1.73	-	5.19 ± 1.13
CYP3A5	28.3 ± 2.7	0.61 ± 0.21	1.64 ± 0.21	5.00 ± 0.52	0.25 ± 0.43	5.08 ± 0.36	2.56 ± 0.21	2.61 ± 0.91	3.81 ± 1.14
CYP3A7	26.3 ± 2.3	-	1.17 ± 0.14	3.50 ± 1.39	-	4.33 ± 1.15	6.14 ± 0.60	-	6.39 ± 0.59

Additional Information

a Major metabolites of human liver microsomes.

b Overall % metabolism of TMC278 calculated from % drug that remained in the sample at the end of the incubation.

No measurable product observed in radio-HPLC profile (LLOQ = 211 dpm).

Report Title	Study Type	Test Article	Report Number
An In Vitro Study to (a) Identify the Microsomal Cytochrome P-450 Iso-enzymes Mediating TMC278 Metabolism (Reaction Phenotyping) and to (b) Determine the Kinetics of TMC278 Metabolism in Human Liver Microsomes	Metabolism, In Vitro	[^{14}C]RPV	TMC278-NC333

Method:
The metabolism of ^{14}C-TMC278 (5 µM) was examined with a characterized panel of 10 human liver microsomal preparations. The protein concentration of the samples was 0.25 mg/ml and the time of incubation was 15 min. The amounts of unchanged TMC278 and its metabolites (M27, M33 [a], M35 + M36 [a] and M42 [a]) were determined by radio-HPLC. The rate of product formation was calculated for TMC278 metabolites and were correlated (pair-wise) with the CYP isoform dependent enzyme activities of corresponding batches of human liver microsomes.

Enzyme activities (CYP isoform)	Overall TMC278 metabolism Correlation (r^2)	TMC278 metabolite correlation coefficient (r^2)			
		M27	M33	M35 + M36	M42
7-ethoxyresorufine *O*-deethylase **(1A2)**	0.119	-0.050	0.523	0.093	0.073
Phenacetin *O*-deethylase **(1A2)**	0.052	-0.186	0.347	0.102	0.047
Coumarin 7-hydroxylase **(2A6)**	-0.071	0.055	-0.297	0.201	-0.080
Taxol 6-α-hydroxylase **(2C8)**	-0.437	-0.613	-0.324	-0.660	-0.289
Tolbutamide methyl hydroxylase **(2C9, 10)**	-0.711	-0.842	-0.530	-0.468	-0.608
S-mephenytoin 4-hydroxylase **(2C19)**	**0.748**	**0.704**	**0.878**	0.107	**0.790**
Dextromethorphan *O*-demethylase **(2D6)**	-0.413	-0.538	-0.310	-0.611	-0.306
Bufuralol hydroxylase **(2D6)**	-0.442	-0.578	-0.348	-0.659	-0.325
Chlorozoxazone 6-hydroxylase **(2E1)**	0.030	-0.098	-0.303	-0.389	0.215
Lauric acid ω-1 hydroxylase **(2E1)**	-0.543	-0.709	-0.393	-0.450	-0.424
Testosterone 6-β-hydroxylase **(3A4)**	**0.819**	**0.749**	0.485	-0.003	**0.881**
Cyclosporine oxidase **(3A)**	**0.716**	**0.744**	0.336	0.015	**0.746**
Taxol 3'- hydroxylase **(3A4)**	**0.889**	**0.938**	**0.503**	0.383	**0.872**
Midazolam 4-hydroxylase **(3A4/A5)**	**0.864**	**0.817**	**0.611**	0.055	**0.876**
Midazolam 1'- hydroxylase **(3A5/A4)**	**0.577**	**0.594**	0.329	-0.310	**0.638**
Lauric acid ω- hydroxylase **(4A)**	-0.001	-0.207	-0.185	-0.499	0.173

a Major metabolite in human liver microsomes.
Bolded numbers: Positive correlations higher than 0.500

8.3. TAF

8.3.1. AD-120-2025: Metabolism of TAF In Vitro (Plasma Stability)

Report Title	Study Type	Test Article	Report Number
In Vitro Metabolism of GS-7340 in Plasma from Dog and Human	Metabolism, In Vitro	TAF	AD-120-2025

Study Systems	Duplicate aliquots of 3 µM TAF were incubated with pooled plasma from beagle dog and human at 37ºC up to 4 hours. Rates of metabolism (in vitro half-life values) were determined. Analysis was done by LC/MS/MS.

Species	Half-Life (min)
Dog	69.5 ± 3.20
Human	74.7 ± 6.40

TAF = tenofovir alafenamide

8.3.2. AD-120-2023: Metabolism of TAF In Vitro in Hepatic S9

Report Title	Study Type	Test Article	Report Number
In Vitro Metabolism of GS-7340 in Hepatic Subcellular Fractions from Dog and Human	Metabolism, In Vitro	TAF	AD-120-2023

Study Systems	Duplicate aliquots of 3 µM TAF were incubated with pooled hepatic S9 fractions from beagle dog and human at 37ºC up to 90 minutes. Rates of metabolism (in vitro half-life values) were determined and hepatic extraction was predicted using the well-stirred liver model. Analysis was done by LC/MS/MS.

Species	Half-Life (min)	Predicted Hepatic Extraction (%)
Dog	31.1 ± 3.40	60.5
Human	20.6 ± 0.70	76.2

TAF = tenofovir alafenamide

8.3.3. AD-120-2024: Metabolism of TAF In Vitro in Intestine S9

Report Title	Study Type	Test Article	Report Number
In Vitro Metabolism of GS-7340 in Intestinal Subcellular Fractions from Dog and Human	Metabolism, In Vitro	TAF	AD-120-2024

Study Systems	Duplicate aliquots of 3 µM TAF were incubated with pooled intestinal S9 fractions from beagle dog and human at 37ºC for up to 120 minutes. Rates of metabolism (in vitro half-life values) were determined. Analysis was done by LC/MS/MS.

Species	Half-Life (min)
Dog	47.1 ± 2.60
Human	58.3 ± 4.40

TAF = tenofovir alafenamide

8.3.4. AD-120-2004: Human Cytochrome P450 Metabolic Reaction Phenotyping of TAF

Report Title	Study Type	Test Article	Report Number
Cytochrome P450 Metabolic Reaction Phenotyping of GS-7340	Metabolism	TAF	AD-120-2004

Methods Rates of metabolism of TAF (5 µM) catalyzed by cDNA expressed major human cytochrome P450 enzyme preparations co-expressed with NADPH CYP450 reductase

Test Compound	Metabolism Rate (min^{-1})					
	CYP1A2	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP3A4
GS-7340 (% Positive Control)	< 0.12 (< 0.9%)	0.23 (< 0.8%)	< 0.47 (< 1.0%)	< 0.12 (< 40%)	< 0.23 (< 0.9%)	1.9 (26.6%)
Ethoxycoumarin	13.2	-	-	-	-	-
Amodiaquine	-	29.3	-	-	-	-
Diclofenac	-	-	47.3	-	-	-
Diazepam	-	-	-	0.29[a]	-	-
Dextromethorphan	-	-	-	-	25.4	-
Testosterone	-	-	-	-	-	7.2

TAF = tenofovir alafenamide; NA = not applicable
a Diazepam is a selective substrate for CYP2C19 but is metabolized slowly

8.3.5. AD-120-2017: Metabolism of TAF In Vitro in Primary Human Hepatocytes

Report Title	Study Type	Test Article	Report Number
In Vitro Activation of GS-1278, GS-4331 and GS-7340 in Primary Human Hepatocytes	Metabolism, In Vitro	TFV, TDF, TAF	AD-120-2017

Study Systems	Duplicate wells of 5 µM test compounds were incubated with primary human hepatocytes for 24 hours. Intracellular concentrations of TFV-DP were determined. Analysis was done by LC/MS/MS.
Sampling Time	24 hours
	TFV-DP Concentration (pmol/million cell)
TFV	12.1
TDF	302
TAF	1,470

TAF = tenofovir alafenamide; TDF = tenofovir disoproxil; TFV = tenofovir; TFV-DP = tenofovir diphosphate

8.3.6. 96-DDM-1278-003: Metabolism of TFV in Dog Plasma, Hepatic S9 and Intestinal S9, and Rat Hepatic Microsomal Fraction

Report Title	Study Type	Test Article	Report Number
In Vitro Metabolism of [^{14}C]PMPA in Human and Animal Tissues	Metabolism, In Vitro	TDF	96-DDM-1278-003

Study Systems Study system 1: rat hepatic microsomal fraction from control animals or following treatment with the general inducer Aroclor 1254.
Study system 2: plasma, intestinal S9 and hepatic S9 from human and dog.

In Vitro Half life (min)
(% TFV remaining following 60 min incubation at 37°C)

	Liver microsomes / S9	Aroclor Hepatic Microsomal Fraction	Plasma	Intestinal S9
Rat	> 60 (117%) [a]	> 60 (93%) [a]	-	-
Rat (+ NADPH)	> 60 (111%) [a]	> 60 (99%) [a]	-	-
Dog	> 60 (92%) [b]	-	> 60 (98%) [b]	> 60 (110%) [b]
Human	> 60 (108%) [b]	-	> 60 (111%) [b]	> 60 (116%) [b]

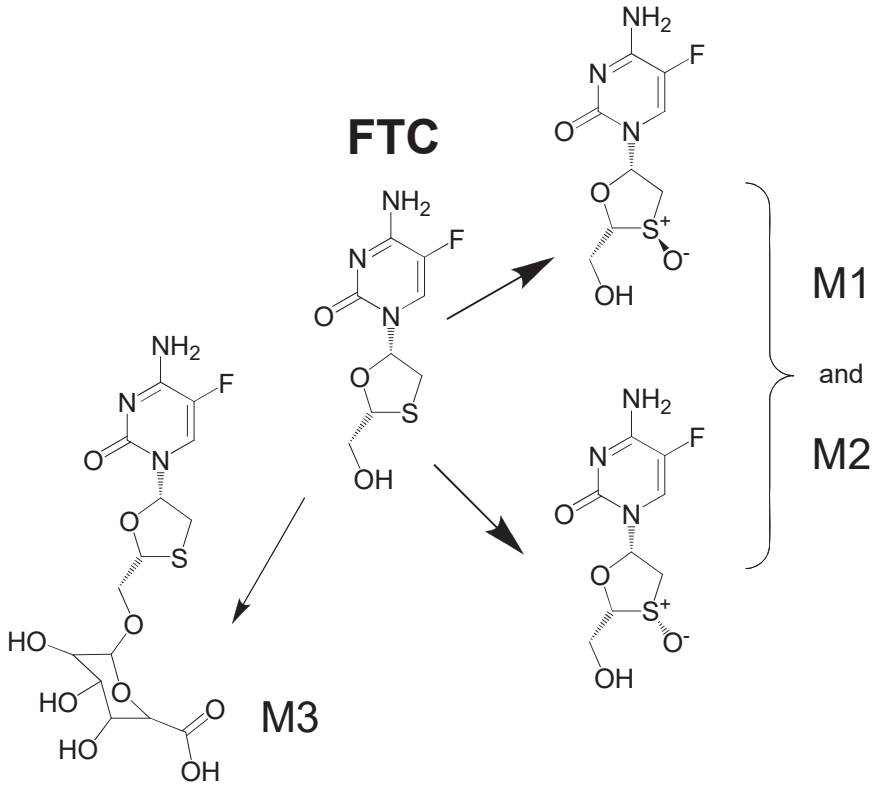
Additional Information: No metabolites were detected.

a Data from study system 1
b Data from study system 2

9. PHARMACOKINETICS: POSSIBLE METABOLIC PATHWAYS

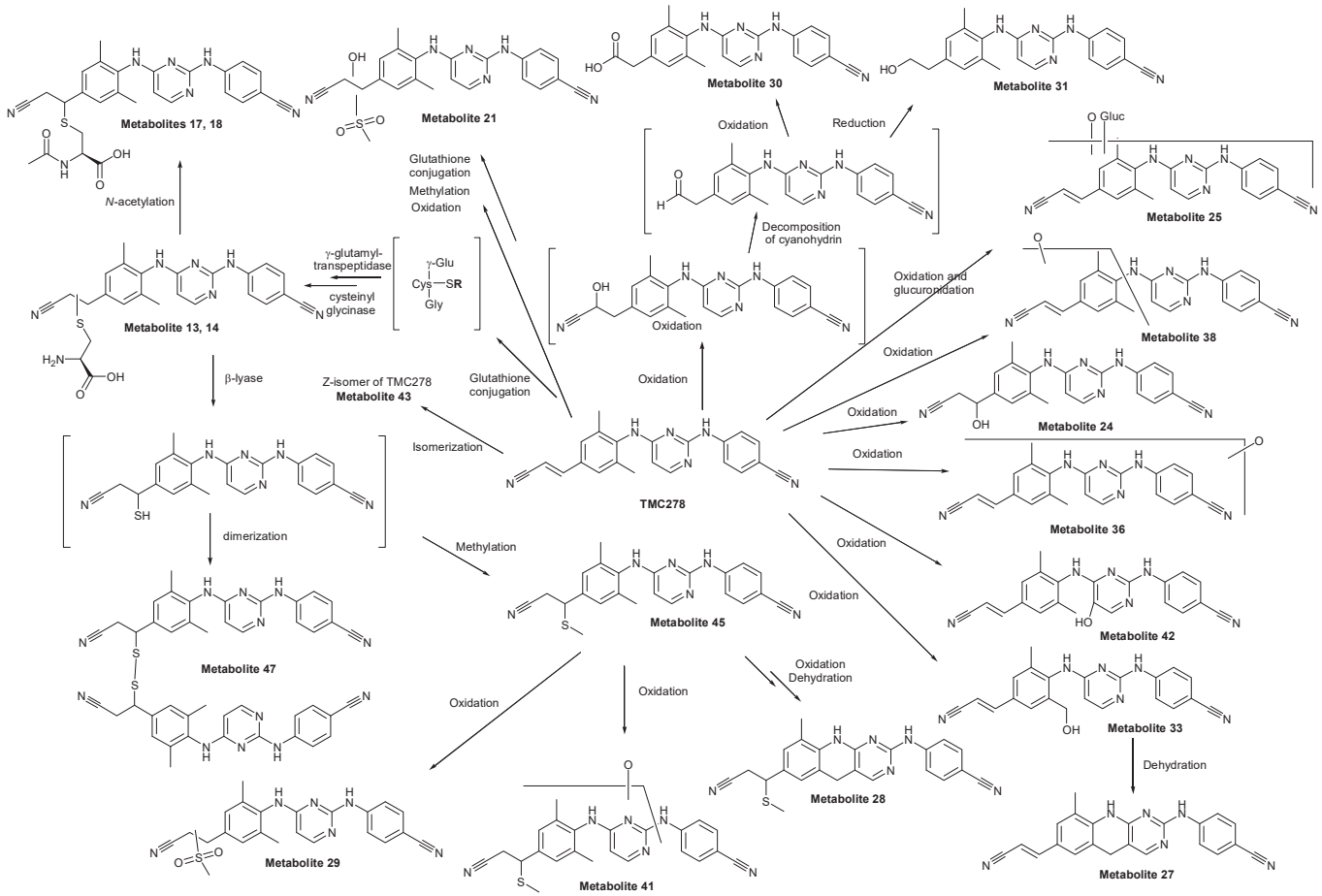
9.1. FTC

The diagram shows the conversion of FTC to its major metabolites: 3'-sulfoxide diastereomers (M1 and M2), and 2'-O-glucuronide (M3).



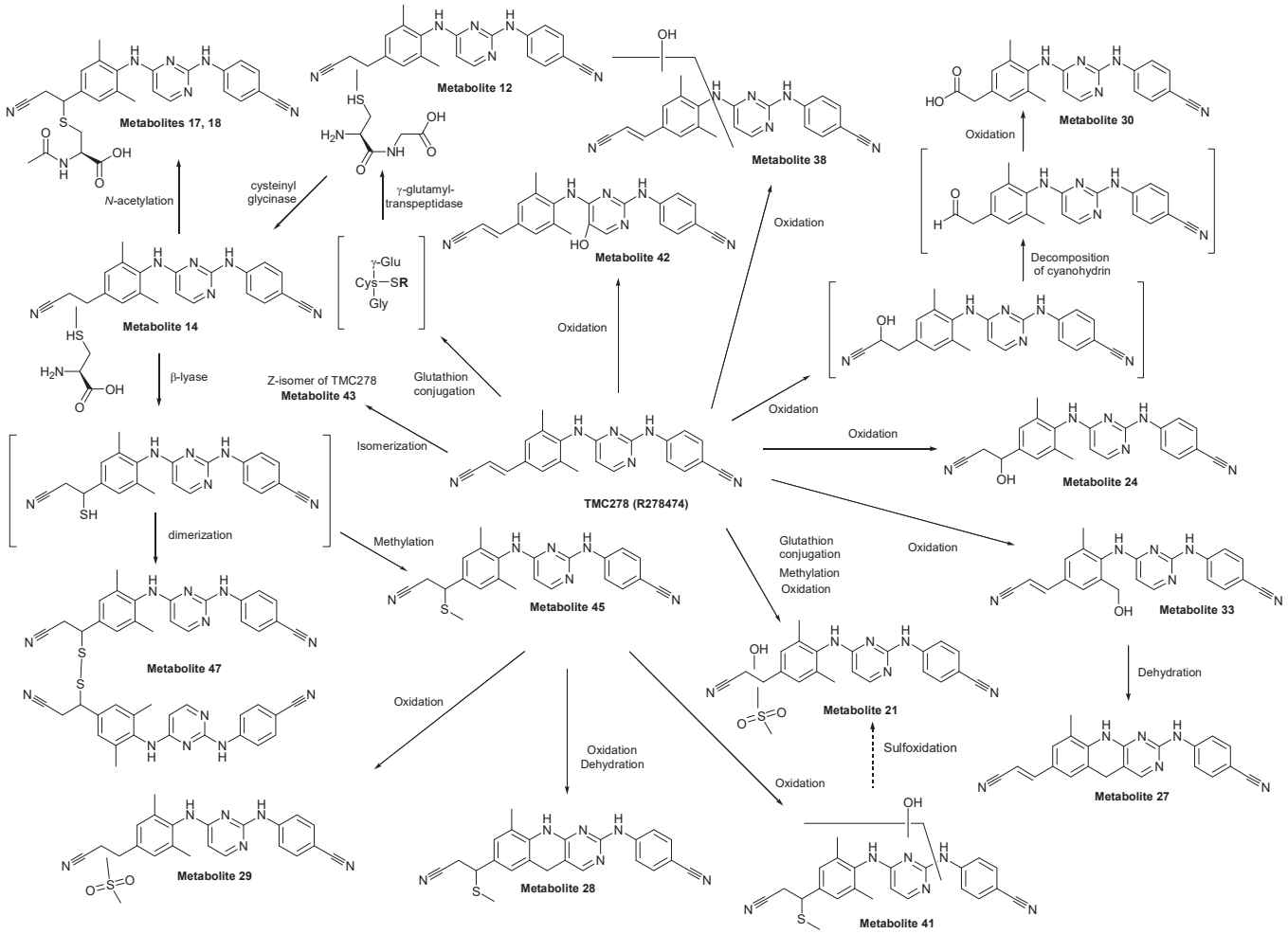
9.2. RPV

9.2.1. RPV Metabolic Pathway in Mouse[a]



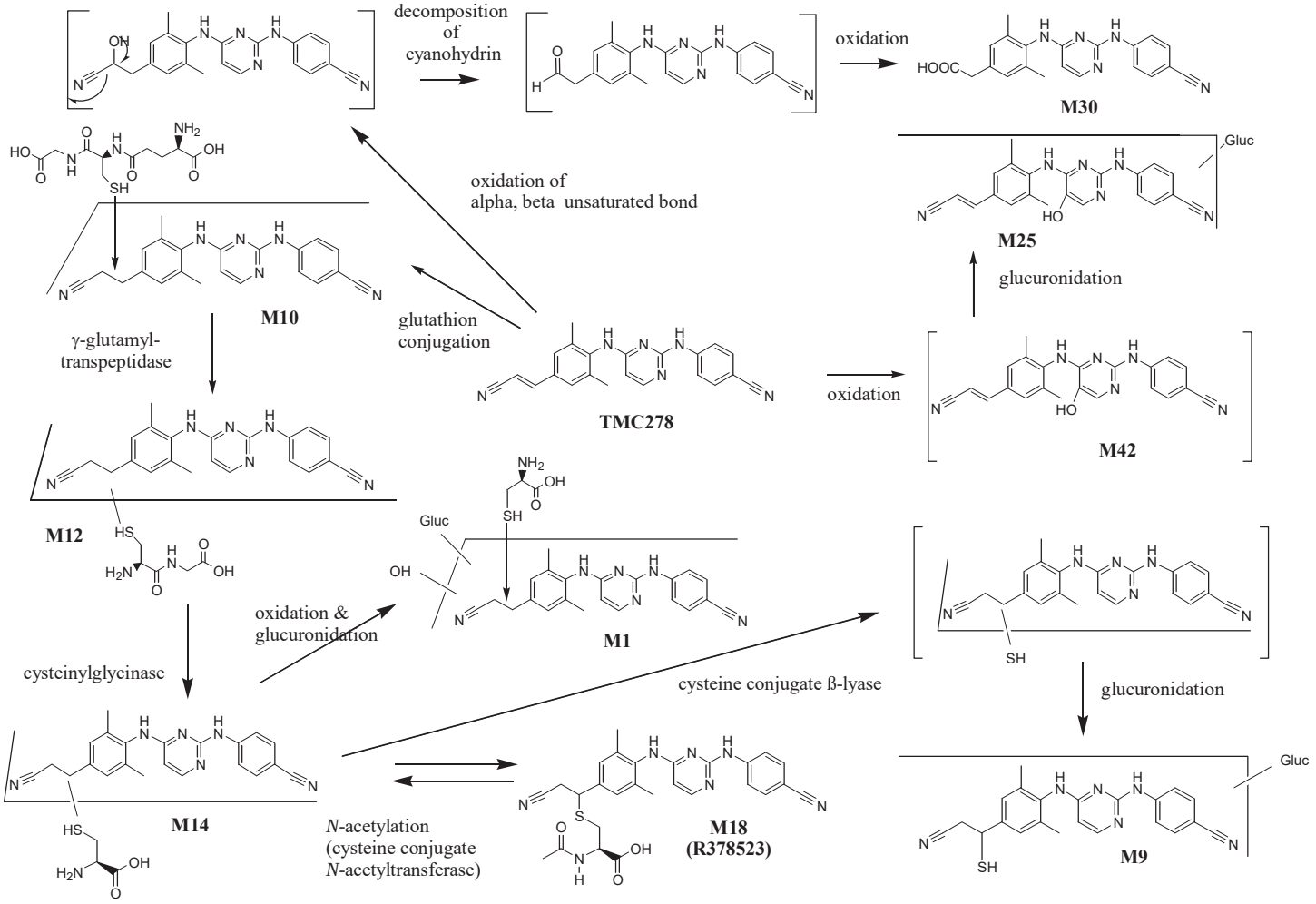
a see Tabulated Summary 10.2.1

9.2.2. RPV Metabolic Pathway in Rat[a]



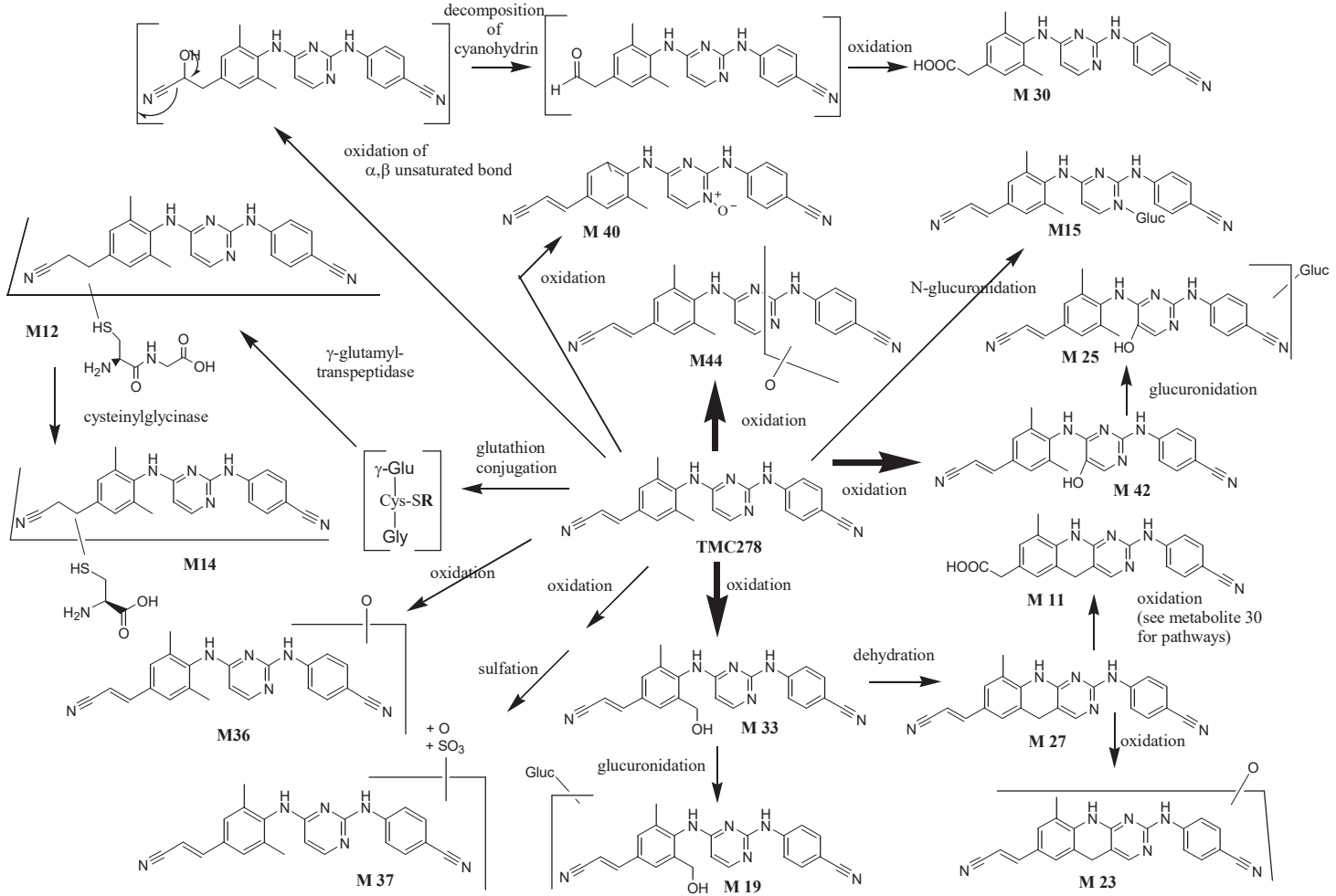
a see Tabulated Summary 10.2.2

9.2.3. RPV Metabolic Pathway in Rat Bile[a]



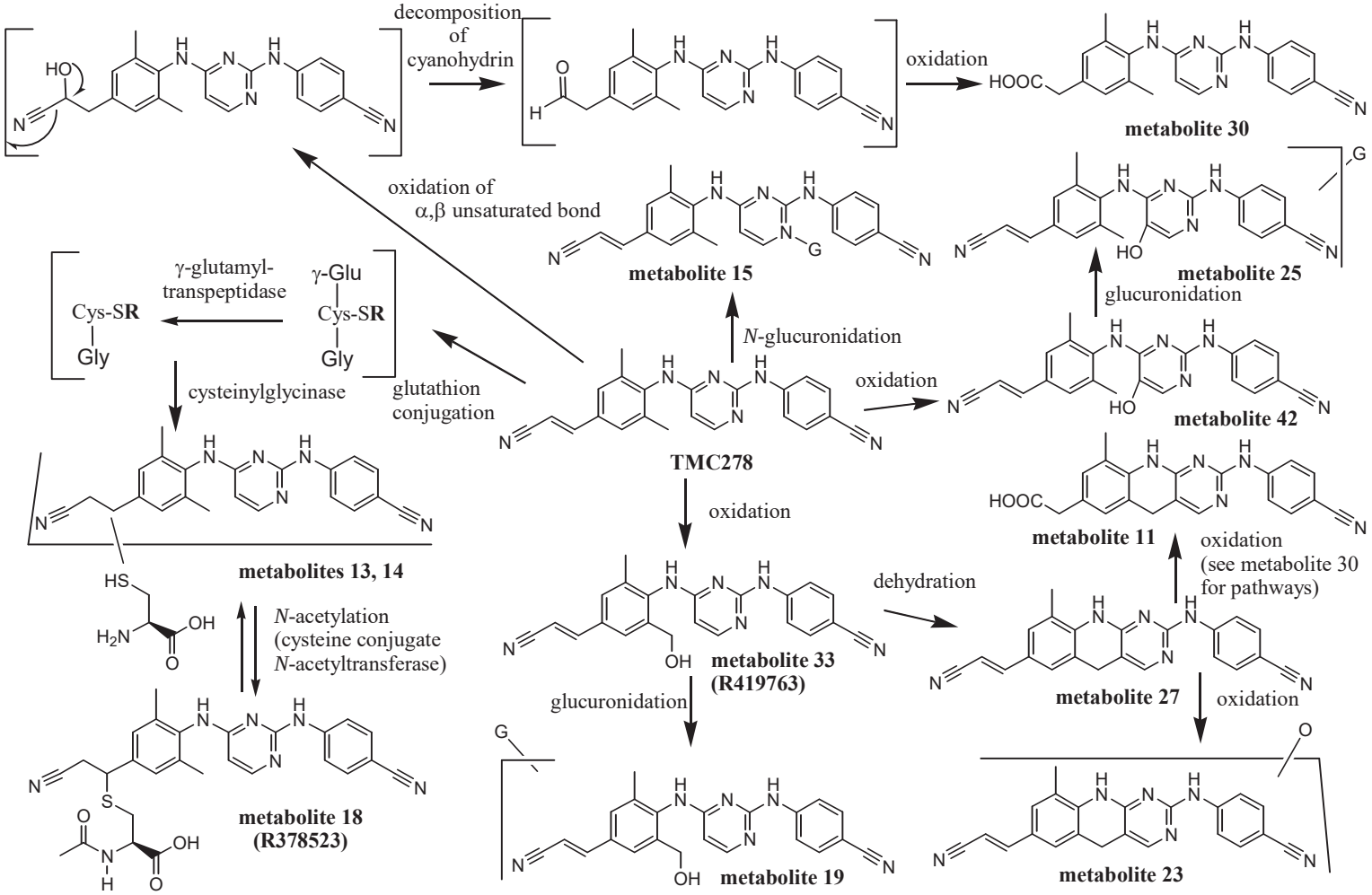
a see Tabulated Summary 10.2.3

9.2.4. RPV Metabolic Pathway in Dog[a]



a see Tabulated Summary 10.2.4

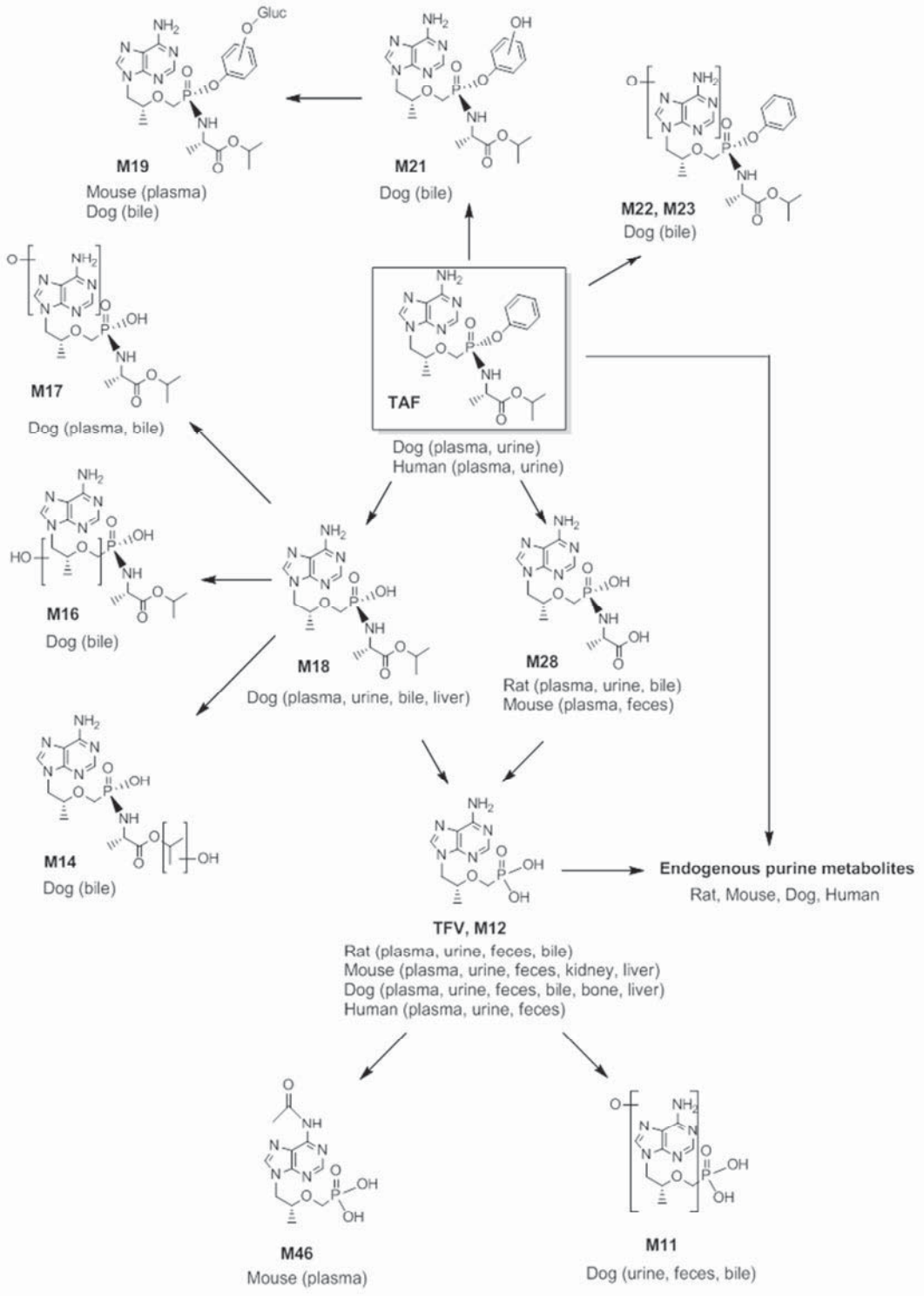
9.2.5. RPV Metabolic Pathway in Human[a]



a see Tabulated Summary 10.2.5

9.3. TAF

The diagram shows TAF metabolites observed in rats, mice, and dogs after a single oral dose of [^{14}C]TAF.



10. PHARMACOKINETICS: METABOLISM IN VIVO

10.1. FTC

10.1.1. TEIN/93/0015: Disposition Study of [^{3}H]FTC in Mice

Report Title	Study Type	Test Article	Report Number
Metabolic Disposition and Balance Studies in Male CD-1 Mice Following Oral Administration of 120 mg/kg [6-^{3}H]524W91	Metabolism, Excretion	FTC	TEIN/93/0015

Species	Mouse, CD-1
Sex (M/F)/Number of Animals	15 M,
Feeding Condition	Not fasted
Vehicle/Formulation	Solution in Water
Method of Administration	Oral
Dose (mg/kg)	120 (single dose)
Radionucleotide	^{3}H
Specific Activity	2.6 μCi/mg

Metabolic Data: Cumulative (0–72 hour postdose) recovery of emtricitabine and its tentatively identified metabolites from the urine of male CD1 mice dosed orally.

Sample	Sampling Time or Period	% of Compound in Sample (Mean ± SD)					
		5-Fluorocytosine	M1/M2[a]	M1/M2[a]	FTC	M3	Unidentified
Urine	0–72 hours	1.4 ± 0.2	1.7 ± 0.3	2.0 ± 0.4	64 ± 7.1	0.5 ± 0.4	0.8 ± 0.2

a Absolute configuration of sulfoxide diastereomers not determined

Report Title	Study Type	Test Article	Report Number
Metabolic Disposition and Balance Studies in Male CD-1 Mice Following Oral Administration of 120 mg/kg [6-^{3}H]524W91	Metabolism, Excretion	FTC	TEIN/93/0015

Excretion Data: The recovery of radioactivity in the urine and feces of male CD1 mice dosed orally.

Sample	Sampling period (hours)	Mean ± SD	
		Percent of dose in sample	Total recovery (%; 0-72 hours)
Urine	0–24	62.3 ± 7.6	66.8 ± 7.0
	24–48	3.4 ± 2.1	
	48–72	1.5 ± 0.4	
Feces	0–24	15.9 ± 3.0	18.1 ± 3.1
	24–48	0.7 ± 0.4	
	48–72	1.5 ± 0.6	
Feces and Urine	0–72	--	85.0 ± 4.2

Additional Information:
Analytical method: Liquid scintillation counting, HPLC radiochromatogram.
Mean ± SD fraction of dose excreted in urine (0–72 hours) as parent drug = 64 ± 7%

10.1.2. TOX063: Metabolism and Excretion of [^{14}C]FTC in Cynomolgus Monkeys

Report Title	Study Type	Test Article	Report Number
Metabolism and excretion of [^{14}C]TP-0006 following oral administration to male Cynomolgus monkeys	Metabolism, Excretion	FTC	TOX063

Species	Monkey, Cynomolgus
Sex (M/F)/Number of Animals	4 M
Feeding Condition	Fasted
Vehicle/Formulation	Sterile water
Method of Administration	Oral
Dose (mg/kg)	200 (single dose)
Radionucleotide	^{14}C
Specific Activity	42.9 µCi/mg and 57.6 µCi/mg

Metabolic Data:

Plasma	**FTC**	**M1/M2**
C_{max} (µg/mL)	46.7	15.9
t_{max}(h)	1	2
$AUC_{0\text{-}last}$ (µg•h/mL)	129	56.6
$AUC_{0\text{-}\infty}$ (µg•h/mL)	133	86.6
CL/F (L/h/kg)	1.57	-

Excretion Data: 40.8% of the administered radioactivity was recovered in the urine, 35.3% in the faeces, and 8.3% in cage washes/wipes. Unchanged parent drug represented the great majority of radioactivity present in urine (approximately 74%) and faeces (97%). The recovery of large amounts of radioactivity in the gut contents following a second dose of radioactivity indicates that much of the faecal recovery represented unabsorbed rather than excreted drug.

Distribution Data: 22 tissues obtained 1 hour postdose. Highest levels: kidneys (596 equivalents µg/g); liver (121 µg/ equivalents /g.); CSF/blood ratio 0.031.

10.1.3. TEIN/93/0016: Metabolism and Excretion of [^{3}H]FTC in Cynomolgus Monkeys

Report Title	Study Type	Test Article	Report Number
Metabolic Disposition of 80 mg/kg Orally Administered [6-3H]524W91 in Cynomolgus Monkeys	Metabolism, Excretion	FTC	TEIN/93/0016

Species	Monkey, cynomolgus
Sex (M/F)/Number of Animals	4 F
Feeding Condition	Fasted overnight and to 2 hours postdose
Vehicle/Formulation	Solution in Water
Method of Administration	Oral
Dose (mg/kg)	80 (single dose)
Radionucleotide	^{3}H
Specific Activity	1.8 μCi/mg

Metabolic Data: Cumulative (0–72 hour postdose) urinary and fecal recovery of FTC (% dose).and its tentatively identified metabolites

	Percent of Dose (Mean ± Standard Deviation)								
Sample	**M200**	**5-Fluorocytosine**	**M1/M2**	**M1/M2**	**M1100**	**M3**	**FTC**	**M1940**	**Deaminated FTC**
Urine	0.2 ± 0.1	0.3 ± 0.04	11 ± 4	1.2 ± 0.2	0.3 ± 0.2	1.6 ± 1.9	28.3 ± 4.1	0.1 ± 0.1	1.1 ± 0.3
Feces	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.03 ± 0.1	--	--	34.5 ± 10.7	--	0.5 ± 0.1

Excretion Data: Examined (pool) samples - the recovery of radioactivity in the urine, feces, and cage washes

		Mean ± Standard Deviation	
	Sampling period (hours)	**Percent of dose in sample**	**Total recovery (% ; 0–72 hours)**
Urine	0–8	32.9 ± 8.6	41.2 ± 6.4
	8–24	5.5 ± 1.1	
	24–48	2.2 ± 1.7	
	48–72	0.6 ± 0.9	
Feces	0–24	23.6 ± 15.9	33.1 ± 10.0
	24–48	4.7 ± 5.7	
	48–72	4.8 ± 9.1	
Cage Wash	0–8	3.0 ± 1.9	9.6 ± 6.7
	8–24	5.0 ± 4.0	
	24–48	0.8 ± 0.6	
	48–72	0.8 ± 0.7	
Overall Recovery	0–72	—	83.8 ± 3.8

10.2. RPV

10.2.1. TMC278-NC190: Metabolism, Excretion and Plasma Kinetics of PV in the Male and Female CD-1 Mouse After Single Oral Administration

Report Title	Study Type	Test Article	Report Number
The Metabolism, Excretion and Plasma Kinetics of TMC278 in the Male and Female CD-1 Mouse After Single Oral Administration of ^{14}CTMC278 at 20 and 320 mg/kg	Metabolism, Excretion	[^{14}C]RPV	TMC278-NC190

Species	Mouse (CD-1)
Gender (M/F)/Number of Animals	M12/F12 (plasma profile) - M12/F12 (excretion mass balance)
Feeding condition	Not fasted
Vehicle/Formulation	PEG400/CA (10%)
Route	Oral
Dose of TMC278 base (mg/kg)	20 and 320
Radionuclide	^{14}C
Specific Activity (kBq/mg)	345 and 23

Report Title		Study Type	Test Article	Report Number
The Metabolism, Excretion and Plasma Kinetics of TMC278 in the Male and Female CD-1 Mouse After Single Oral Administration of 14CTMC278 at 20 and 320 mg/kg		Metabolism, Excretion	RPV	TMC278-NC190

Dose (mg/kg)		20				320			
Sample		Urine		Fecal Extract		Urine		Fecal Extract	
		Male	Female	Male	Female	Male	Female	Male	Female
Time (h)		0-24	0-24	0-48	0-48	0-24	0-24	0-48	0-48
% of Administered Radioactivity in Excreta		**2.82**	**3.46**	**64.05**	**65.38**	**1.27**	**2.88**	**75.2**	**71.3**
Parent (UD)		0.63	0.08	8.2	7.8	0.11	0.02	33	34
M13	Cysteine-S-conjugate	0.14	0.39			0.09	0.31		
M14	Cysteinyl-S-conjugate	0.08	0.58			0.06	0.55		
M13+M14				5.0	4.4			5.1	2.8
M17	Mercapturic acid conjugate	0.19	0.15			0.08	0.14		
M18	Mercapturic acid conjugate	0.13	0.07			0.04	0.10		
M17+M18				4.2	2.3			3.3	3.4
M21	Hydroxylated sulfonyl conjugate			1.4	1.0			0.7	0.3
M25	Oxidation combined with glucuronidation	0.42	1.6			0.42	1.6		
M24+M25	Hydroxylation on the cyanoethenyl moiety, Oxidation combined with glucuronidation			3.4	2.7			1.6	1.6
M27+M28+M29	Dehydration of M33; Aliphatic hydroxylation and dehydration of M45; Sulphoxidation of M45			0.3	<0.2			<0.2	0.1
M30	Carboxylic acid metabolite			1.6	3.1			1.5	1.2
M33	Aliphatic hydroxylation			0.5	0.7			1.3	1.0
M35	Unknown structure			<0.2	<0.2			<0.2	<0.2
M38	Hydroxylation			1.4	1.4			1.0	0.8
M41+M42[a]	Hydroxylation of M45; Aromatic hydroxylation			18	26			9.2	13
M42	Aromatic hydroxylation	0.37	0.20			0.11	0.06		

Report Title		Study Type	Test Article	Report Number
The Metabolism, Excretion and Plasma Kinetics of TMC278 in the Male and Female CD-1 Mouse After Single Oral Administration of 14CTMC278 at 20 and 320 mg/kg		Metabolism, Excretion	RPV	TMC278-NC190

Dose (mg/kg)		20				320			
Sample		Urine		Fecal Extract		Urine		Fecal Extract	
		Male	Female	Male	Female	Male	Female	Male	Female
Time (h)		0-24	0-24	0-48	0-48	0-24	0-24	0-48	0-48
M43+M45	Z-isomerization , S-Methyl conjugate			2.2	1.8			1.3	1.2
M46	Unknown structure			0.5	0.7			0.5	0.6
M47	Dimerization			0.3	<0.2			0.8	0.2

a Metabolite fraction mainly composed of M42, estimated at about 14% and 17% of the 20 mg/kg dose, and at 5.9% and 8.0% of the 320 mg/kg dose in male and female mice, respectively

CA: citric acid; F: female; M: male; PEG400: polyethylene glycol; UD: unchanged drug

Report Title		Study Type	Test Article	Report Number
The Metabolism, Excretion and Plasma Kinetics of TMC278 in the Male and Female CD-1 Mouse After Single Oral Administration of 14CTMC278 at 20 and 320 mg/kg		Metabolism, Excretion	RPV	TMC278-NC190

Dose (mg/kg)		**20**											
Sample		**Plasma**											
		Male			**Female**			**Male**			**Female**		
Time (h)		**1**	**3**	**8**	**1**	**3**	**8**	**1**	**3**	**8**	**1**	**3**	**8**
% of Plasma radioactivity		**11.1** [b]	**6.85** [b]	**9.46** [b]	**8.28** [b]	**5.70** [b]	**12.5** [b]	**30.7** [b]	**26.3** [b]	**42.5** [b]	**26.8** [b]	**32.2** [b]	**49.4** [b]
Parent (UD)		105	98	97	99	97	95	93	86	95	95	95	91
M13+M14	Cysteine-S-conjugate; Cysteinyl-S-conjugate	0.5	0.5	0.6	0.5	<0.4	0.6	<0.8	0.9	1.0	<1.2	1.8	<0.8
M27	Dehydration of M33	0.6	0.8	0.8	0.6	0.9	0.6	<0.8	1.2	<0.8	<1.2	<0.7	<0.8
M30	Carboxylic acid metabolite	0.6	0.5	0.6	0.7	0.8	1.0	<0.8	0.9	1.4	<1.2	1.3	1.5
M33	Aliphatic hydroxylation	4.5	3.1	3.5	3.4	6.6	4.3	2.7	2.1	1.5	2.8	4.1	2.2
M36	Hydroxylation of TMC278	0.3	0.8	0.4	0.6	1.3	0.5	<0.8	<0.9	<0.8	1.2	<0.7	<0.8

Additional Information
96 h after administration of TMC278, 88% and 87% of the dose was excreted in feces and 3.5% and 4.2% in urine at 20 mg/kg and 96% and 89% of the dose was excreted in feces and 1.84% and 3.6% in urine at 320 mg/kg, in males and females, respectively. Over the 4-day collection period, more than 95% of the administered dose was recovered.
b Total radioactivity levels in µg-eq./mL.
CA: citric acid; F: female; M: male; PEG400: polyethylene glycol; UD: unchanged drug

10.2.2. TMC278-NC113: Metabolism and Excretion of ^{14}C-RPV in the Male and Female Sprague-Dawley Rat After Single Oral Administration

Report Title	Study Type	Test Article	Report Number
The Metabolism and Excretion of ^{14}C-TMC278 in the Male and Female Sprague-Dawley Rat After Single Oral Administration of ^{14}CTMC278 at 40 mg/kg	Metabolism, Excretion	[^{14}C]RPV	TMC278-NC113

Species	Rat (Sprague Dawley)
Gender (M/F)/Number of Animals	M12/F12 (plasma profile) – M5/F5 (excretion mass balance)
Feeding condition	Not fasted
Vehicle/Formulation	TMC278 base in PEG400/CA (10%)
Route	Oral
Dose of TMC278 base (mg/kg)	40
Radionuclide	^{14}C
Specific Activity (kBq/mg)	37

Report Title		Study Type	Test Article	Report Number
The Metabolism and Excretion of ^{14}C-TMC278 in the Male and Female Sprague-Dawley Rat After Single Oral Administration of ^{14}CTMC278 at 40 mg/kg		Metabolism, Excretion	[^{14}C]RPV	TMC278-NC113

Sample		Urine		Fecal Extract		Plasma					
		Male	Female	Male	Female	Male			Female		
Time (h)		0-24	0-24	0-48	0-48	1	4	8	1	4	8
% of Administered Radioactivity in Excreta or µg-eq./mL in Plasma		0.39[a)]	1.6[a)]	74[a)]	65[a)]	1.4	1.1	0.88	4.0	3.7	2.0
Parent (UD)		n.d.	0.01	47	43	60[b)]	72[b)]	71[b)]	83[b)]	78[b)]	64[b)]
M12+M14	Cysteinylglycine-S-conjugate; Cysteinyl-S-conjugate	n.d.	n.d.	n.d.	n.d.	7.0[c)]	3.8[c)]	5.6[c)]	14[c)]	13[c)]	10[c)]
M17	Mercapturic acid conjugate	0.02	1.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M18	Mercapturic acid conjugate	0.03	0.45	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M21	Hydroxylated sulfonyl conjugate	n.d.	n.d.	0.20	0.44	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M24+M27+M28+ M29	Hydroxylation on cyanoethenyl moiety; Dehydration of M33; Aliphatic hydroxylation and dehydration of M45; Oxidation of M45	n.d.	n.d.	0.99[d)]	1.6[d)]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M30	Carboxylic acid metabolite	n.d.	n.d.	0.47	0.05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M33	Aliphatic hydroxylation	n.d.	n.d.	0.54	0.54	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M38	Hydroxylation	n.d.	n.d.	0.70	0.82	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M41+M42	Hydroxylation of M45; Aromatic hydroxylation	n.d.	n.d.	2.8[e)]	3.6[e)]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M43+M45	Isomerization; S-Methyl conjugate	n.d.	n.d.	2.4[f)]	2.9[f)]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M46	Unknown	n.d.	n.d.	0.99	0.92	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M47	Dimerization	n.d.	n.d.	4.0	3.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Additional Information

a Percentage of administered dose.b Percentage of injected sample radioactivity. c Sum of % of administered dose of M12 and M14, which co-eluted together in rat plasma samples.

d Sum of % of administered dose of M24, M27, M28 and M29, which co-eluted together in rat feces samples. e Sum of % of administered dose of M41 and M42, which co-eluted together in rat feces samples.

f Sum of % of administered dose of M43 and M45, which co-eluted together in rat feces samples.

n.d. – Not detected in sample or under the limit of quantification. 96 h after administration of TMC278, 93% of the dose was excreted in feces and 0.45% and 1.8% in urine in males and females, respectively. Over the 4-day collection period, 94% and 95% of the administered dose was recovered in males and females, respectively.

10.2.3. TMC278-NC145: Biliary Metabolism and Excretion of RPV in Male Sprague-Dawley Rats After Oral Dose

Report Title	Study Type	Test Article	Report Number
Biliary Excretion and Identification of Biliary Metabolites of TMC278 in Male Sprague-Dawley Rats After a Single Oral Dose of 14C-TMC278 at 40 mg/kg	Metabolism	[^{14}C]RPV	TMC278-NC145

Species	Rat (Sprague Dawley)
Gender (M/F)/Number of Animals	M/3 (restrained); M/3 (non-restrained)
Feeding condition	Not fasted
Vehicle/Formulation	TMC278 base in PEG400/CA (10%)
Route	Oral
Dose of TMC278 base (mg/kg)	40
Radionuclide	^{14}C
Specific Activity (kBq/mg)	37

Sample		**Bile (Restrained Rats)**	**Bile (Non-Restrained Rats)**
Time (h)		**0-24**	**0-24**
% of Administered Radioactivity in Bile		**18***	**25**
Metabolite Code		**Metabolite Profile - % Dose Radioactivity in 0-24 h Bile**	**Samples Not Profiled**
Parent (UD)		0.19	
M1	Glucuronidation and oxidation of M14	0.63	
M9	Thiol glucuronide conjugate	2.8	
M10+M12+M14	Cysteinylglycine-S-conjugate; Cysteinyl-S-conjugate	6.4	
M18	Mercapturic acid conjugate	2.7	
M25	Oxidation combined with glucuronidation	1.4	
M30	Carboxylic acid metabolite	0.4	
	Sum	**14***	

* The calculated total % dose value (14%) varies from the actual value (18%) due to an error arising from integration of noise in a radiochromatogram.

10.2.4. TMC278-NC114: Absorption, Metabolism and Excretion of RPV in the Male Beagle Dog after Oral Dose

Report Title	Study Type	Test Article	Report Number
The Absorption, Metabolism and Excretion of TMC278 in the Male Beagle Dog after a Single Oral Dose of ^{14}C-TMC278 at 5 mg/kg	Metabolism	[^{14}C]RPV	TMC278-NC114

Species	Dog (beagle)
Gender (M/F)/Number of Animals	3M
Feeding condition	Fed
Vehicle/Formulation	TMC278 base in PEG400/CA (10%)
Route	Oral (Capsule)
Dose of TMC278 base (mg/kg)	5
Radionuclide	^{14}C
Specific Activity (kBq/mg)	99

Report Title		**Study Type**	**Test Article**	**Report Number**
The Absorption, Metabolism and Excretion of TMC278 in the Male Beagle Dog after a Single Oral Dose of ^{14}C-TMC278 at 5 mg/kg		Metabolism	RPV	TMC278-NC114

Sample		**Urine**	**Feces**	**Plasma**							
Time (h)		**0-168h**	**0-72**	**0.25**	**0.5**	**1**	**2**	**4**	**6**	**8**	**24**
% of Administered Radioactivity in Excreta or µg-eq./mL in Plasma		1.73	81	0.06	0.21	0.37	0.57	0.65	0.54	0.46	0.40
Pooled Sample Results: % Dose Recovered		**0-24 h**	**0-72 h**	**Pooled plasma[a)]**		**1h**		**4h**		**8h**	
Parent (UD)		n.d	45			94		73		91	
M11	Carboxylic acid metabolite on the cyanoethenyl moiety of M27	n.d	0.98			n.d.		n.d.		n.d.	
M23+M27	Monooxy-M27; Tricyclic metabolite, originating from oxidation and dehydration	n.d	2.1			n.d.		n.d.		n.d.	
M30+M48	Carboxylic acid metabolite; Unknown	0.03, n.d.	3.1			n.d.		n.d.		n.d.	
M33	Aliphatic hydroxylation	n.d	8.7			n.d.		n.d.		n.d.	
M37	Sulfate conjugate of monooxygenated-TMC278	n.d	0.31			n.d.		n.d.		n.d.	
M40	N-oxide-TMC278	n.d	0.50			n.d.		n.d.		n.d.	
M42	Aromatic hydroxylation	n.d	5.3			n.d.		n.d.		n.d.	
M44	Monooxygenated TMC278	n.d	4.3			n.d.		n.d.		n.d.	
M46	Unknown structure	n.d	0.09			n.d.		n.d.		n.d.	
M49	Unknown structure	n.d	1.4			n.d.		n.d.		n.d.	

Additional Information
a Percentage of injected sample radioactivity.
n.d. = Not detected by radiometric detector.
In urine, minor metabolites (M3, M12, M14, M19, M25 and M36) were detected which accounted for less than 0.08% of the dose. In plasma, minor metabolites were present in trace amounts and included M15, M19, M27, M30, and M33
168 h after administration of TMC278, 95% and 1.7% of the administered dose was excreted in feces and urine, respectively. Over the 7-day collection period, 97% of the administered dose was recovered.

10.2.5. TMC278-NC157: Absorption, Metabolism and Excretion of TMC278 After a Single Oral Dose of 150 mg in Healthy Male Subjects

Report Title	Study Type	Test Article	Report Number
The Absorption, Metabolism and Excretion of TMC278 After a Single Oral Dose of 150 mg in Healthy Male Subjects (Clinical Trial TMC278-C119)	Metabolism, Excretion	[^{14}C]RPV	TMC278-NC157

Species	Male Subjects
Gender (M/F)/Number of Subjects	6M
Feeding condition	Fed (Breakfast)
Vehicle/Formulation	TMC278 base in PEG400 (25 mg/mL)
Route	Oral
Dose of TMC278 (mg)	150
Radionuclide	^{14}C
Specific Activity (kBq/mg)	11.8

Report Title	Study Type	Test Article	Report Number
The Absorption, Metabolism and Excretion of TMC278 After a Single Oral Dose of 150 mg in Healthy Male Subjects (Clinical Trial TMC278-C119)/Three-Month Repeated Dose Oral Toxicity Study in the Swiss Mouse	Metabolism	[^{14}C]RPV	TMC278-NC157

Sample		**Urine**	**Feces**	**Plasma Total Radioactivity (% of Sample Radioactivity in plasma)**					
Time (h)		**0-168h**	**0-168h**	**1h**	**2h**	**4h**	**8h**	**12h**	**24h**
% of Administered Radioactivity or Conc. (µg-eq./mL)		6.13	76	0.40	0.57	0.77	0.56	0.36	0.32
Average Sample Results: % Dose Recovered			**Selection of Samples**	**1h**	**2h**	**4h**	**8h**	**12h**	**24h**
Parent (UD)		Traces	25.5	58.8	55.7	65.7	47.2	45.7	44.1
M3	Unknown structure		0.3						
M11	Carboxylic Acid Metabolite of M27		1.6						
M13+M14 and M18	Glycine conjugates of TMC278; mercapturic acid conjugate of TMC278	1.2							
M15	*N*-Glucuronide of TMC278	0.6		4.2	4.1	5.3	8.7	9.5	6.2
M19	Glucuronide of M33	0.3							
M23	Oxidized Metabolite of M27		0.7						
M25	Glucuronide of M42	0.6							
M27	Tricyclic Metabolite		0.6	6.6	6.6	9.7	6.5	5.7	8.0
M30	Carboxylic Acid Metabolite	0.03	2.7						
M33	Hydroxymethyl TMC278		3.0	LOQ	1.8	2.9	3.4	3.5	5.1
M35	Unknown structure		2.2						
M39	Cis 5-Hydroxy Pyrimidinyl (Cis of M42)		0.4						
M42	5-Hydroxyl Pyrimidinyl		16.1						
M43	Cis TMC278		0.6						
M46	Unknown structure		0.5						

Additional Information

a Expressed as percent of the administered dose in the 0-168h urine and 0-336h faeces.

LOQ = Below the limit of quantification. 168 h after administration of TMC278, 76% and 6.1% of the administered dose was excreted in feces and urine, respectively. Over the 14-day collection period, 91% of the administered dose was recovered.

10.3. TAF

10.3.1. AD-120-2012: Metabolism of TAF in Mouse

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Feces, Kidney, Liver, and Nasal Turbinate Samples from Mice after Oral Administration of [^{14}C]GS-7340 and Stability of [^{14}C]GS-7340 in vitro using CD-1 Mouse Hepatic Microsomes and Plasma	Metabolism	[^{14}C]TAF	AD-120-2012

Methods:

Species	CD-1 mice
Sex (M/F) / No. of Animals	Male/30
Method of Administration:	Oral gavage
Dose (mg/kg):	100
Feeding Condition:	Not fasted
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample	Plasma
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

Final Metabolite Designation	Concentrations (ng Equivalents [^{14}C]GS-7340/g)					AUC_{0-24h} (ng eq•h/g)	% AUC of Total Radioactivity
	0.25 h	1 h	2 h	12 h	24 h		
M27A (Allantoin)	549	420	1820	143	121	10530	12.2
M27B (Uric acid)	259	1150	1420	390	371	16670	19.4
M5	1050	239	277	73.4	ND	2411	2.80
M6	280	86.4	160	ND	ND	NA	NA
M28	1690	425	169	ND	ND	878	1.02
M12	13600	4740	5030	1340	385	47167	54.8
M45	2260	667	354	ND	ND	3177	3.69
M46	2740	688	326	ND	ND	2738	3.18
M18	ND	ND	201	ND	ND	NA	NA
M47	ND	ND	166	ND	ND	NA	NA
M19	332	20.9	765	ND	ND	1533	1.78
M23	ND	ND	125	ND	ND	NA	NA

NA = not applicable; ND = peak was not detected or below the established limit of quantitation; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Feces, Kidney, Liver, and Nasal Turbinate Samples from Mice after Oral Administration of [^{14}C]GS-7340 and Stability of [^{14}C]GS-7340 in vitro using CD-1 Mouse Hepatic Microsomes and Plasma	Metabolism	[^{14}C]TAF	AD-120-2012

Methods:

Species CD-1 mice

Sex (M/F) / No. of Animals Male/30

Method of Administration: Oral gavage

Dose (mg/kg): 100

Feeding Condition: Not fasted

Vehicle Formulation water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Analyte/Assay: [^{14}C]TAF/Liquid Scintillation Counter

	Urine (% Radioactive Dose)				Feces (% Radioactive Dose)			
	Collection Interval (h)				Collection Interval (h)			
Final Metabolite Designation	0-24	24-48	48-72	Total	0-24	24-48	48-72	Total
M27A (Allantoin)	1.56	0.70	0.32	2.57	0.42	ND	0.02	0.43
M5	1.91	0.23	0.02	2.16	NA	NA	NA	NA
M12	13.0	4.12	0.99	18.1	25.3	4.14	1.26	30.7
M28	NA	NA	NA	NA	0.67	ND	ND	0.67
M32	0.36	ND	ND	0.36	NA	NA	NA	NA
M35	0.66	ND	ND	0.66	NA	NA	NA	NA

NA = not applicable; ND = peak was not detected or below the established limit of quantitation; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Feces, Kidney, Liver, and Nasal Turbinate Samples from Mice after Oral Administration of [^{14}C]GS-7340 and Stability of [^{14}C]GS-7340 in vitro using CD-1 Mouse Hepatic Microsomes and Plasma	Metabolism	[^{14}C]TAF	AD-120-2012

Methods:

Species CD-1 mice

Sex (M/F) / No. of Animals Male/30

Method of Administration: Oral gavage

Dose (mg/kg): 100

Feeding Condition: Not fasted

Vehicle Formulation water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Analyte/Assay: [^{14}C]TAF/Liquid Scintillation Counter

	Kidney Concentration (ng equivalents [^{14}C]GS-7340/g)			**Liver Concentration (ng equivalents [^{14}C]GS-7340/g)**		
	Collection Time (h)			**Collection Time (h)**		
Final Metabolite Designation	**1**	**24**	**48**	**1**	**24**	**48**
M27A (Allantoin)	NA	NA	NA	107000	63800	35900
M27B (Uric acid)	4250	3050	2180	NA	NA	NA
M7 (Xanthine)	2830	2070	2460	NA	NA	NA
M8 (Hypoxanthine)	1010	731	1190	NA	NA	NA
M12	90300	18000	4620	356000	93200	18500

NA = not applicable; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Feces, Kidney, Liver, and Nasal Turbinate Samples from Mice after Oral Administration of [^{14}C]GS-7340 and Stability of [^{14}C]GS-7340 in vitro using CD-1 Mouse Hepatic Microsomes and Plasma	Metabolism	[^{14}C]TAF	AD-120-2012

Methods:

Species CD-1 mice

Sex (M/F) / No. of Animals Male/30

Method of Administration: Oral gavage

Dose (mg/kg): 100

Feeding Condition: Not fasted

Vehicle Formulation water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Analyte/Assay: [^{14}C]TAF/Liquid Scintillation Counter

	Concentration (ng equivalents [^{14}C]GS-7340/g)				
	Collection Time (h)				
Final Metabolite Designation	**1**	**4**	**12**	**24**	**48**
M1	392	214	250	325	399
M2	794	326	364	ND	ND
M5	ND	ND	ND	90.6	ND
M7 (Xanthine)	953	ND	ND	46.8	ND
M8 (Hypoxanthine)	254	121	342	298	373
M29	2060	737	599	254	ND
M30	159	ND	164	213	136
M34	ND	ND	ND	70.1	106

ND = peak was not detected or below the established limit of quantitation; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Feces, Kidney, Liver, and Nasal Turbinate Samples from Mice after Oral Administration of [^{14}C]GS-7340 and Stability of [^{14}C]GS-7340 in vitro using CD-1 Mouse Hepatic Microsomes and Plasma	Metabolism	[^{14}C]TAF	AD-120-2012

Study Systems [^{14}C]GS-7340 (5 µM) was incubated at 37°C with either pooled plasma or hepatic microsomes (with 1 mMNADPH) from male CD-1 mice for 0 and 60 minutes. The extracted samples were analyzed by HPLC with in-line radioactivity detection to determine the metabolite profiles.

Final Metabolite Designation	Plasma		Hepatic Microsomes (1 mg/mL protein)	
	Percent of Radioactivity Injected (% of Run)		Percent of Radioactivity Injected (% of Run)	
	0 minute	60 minute	0 minute	60 minute
[^{14}C]M1	NA	NA	ND	0.77
[^{14}C]M2	ND	43.0	3.00	11.1
[^{14}C]M5	NA	NA	ND	ND
[^{14}C]M6	NA	NA	ND	ND
[^{14}C]M28	ND	46.0	2.24	3.98
[^{14}C]M29	NA	NA	ND	0.72
[^{14}C]M30	NA	NA	ND	0.82
[^{14}C]M31	NA	NA	ND	0.35
[^{14}C]M32	ND	5.70	NA	NA
[^{14}C]M33 ([^{14}C]Adenine)	NA	NA	ND	41.0
[^{14}C]M18	43.6	0.87	1.63	0.89
[^{14}C]M20	28.5	ND	ND	ND
[^{14}C]M36	NA	NA	3.35	1.38
[^{14}C]M37	NA	NA	ND	0.70
[^{14}C]M38	NA	NA	ND	3.08
[^{14}C]M39	NA	NA	ND	1.87
[^{14}C]M40	NA	NA	ND	2.96
[^{14}C]M41	NA	NA	ND	0.41
[^{14}C]M42	NA	NA	ND	0.50
[^{14}C]GS-7340	24.1	ND	84.1	16.3

NA = not applicable; ND = peak was not detected or below the established limit of quantitation; TAF = tenofovir alafenamide

10.3.2. AD-120-2021: Metabolism of TAF in Rat

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Rats after Oral Administration of [^{14}C]GS-7340	Metabolism	[^{14}C]TAF	AD-120-2021

Methods:

Species	Sprague-Dawley rats
Sex (M/F) / No. of Animals	Male/15
Method of Administration:	Oral gavage
Dose (mg/kg):	5
Feeding Condition:	Not fasted
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample	Plasma
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

Final Metabolite Designation	Plasma Concentrations (ng Equivalents [^{14}C]GS-7340/g)						AUC_{0-24h} (ng eq•h/g)	% AUC of Total Radioactivity
	0.25 h	0.5 h	1 h	2 h	12 h	24 h		
M27A (Allantoin)	425	259	42.4	8.55	ND	ND	634	23.2
M28	221	135	15.0	ND	ND	ND	159	5.80
M12	246	264	99.7	58.8	19.4	ND	1153	66.7

ND = peak was not detected or below the established limit of quantitation; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Rats after Oral Administration of [^{14}C]GS-7340	Metabolism	[^{14}C]TAF	AD-120-2021

Methods:

Species	Sprague-Dawley rats
Sex (M/F) / No. of Animals	Male/3
Method of Administration:	Oral gavage
Dose (mg/kg):	5
Feeding Condition:	Not fasted
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample	Urine
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

	Urine (Percent of Radioactive Dose)	Feces (Percent of Radioactive Dose)		
	Collection Interval (h)	Collection Interval (h)		
Final Metabolite Designation	**0-24**	**0-24**	**24-48**	**Total**
M27A (Allantoin)	0.14	NA	NA	NA
M44	1.41	NA	NA	NA
M28	ND	NA	NA	NA
M12	17.1	59.8	3.56	63.3
M32	0.19	NA	NA	NA

NA = not applicable; ND = peak was not detected or below the established limit of quantitation; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Rats after Oral Administration of [^{14}C]GS-7340	Metabolism	[^{14}C]TAF	AD-120-2021

Methods:

Species	Bile Duct-Cannulated Sprague-Dawley rats
Sex (M/F) / No. of Animals	Male/3
Method of Administration:	Oral gavage
Dose (mg/kg):	5
Feeding Condition:	Not fasted
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

	Urine (Percent of Radioactive Dose)	Bile (Percent of Radioactive Dose)	Feces (Percent of Radioactive Dose)		
	Collection Interval (h)		Collection Interval (h)		
Final Metabolite Designation	**0-24**	**0-4**	**0-24**	**24-48**	**Total**
M27A (Allantoin)	0.23	NA	NA	NA	NA
M27B (Uric acid)	NA	0.02	NA	NA	NA
M44	1.38	NA	NA	NA	NA
M28	0.38	1.17	NA	NA	NA
M12	17.1	0.67	49.3	12.4	61.7
M32	0.30	NA	NA	NA	NA

NA = not applicable; TAF = tenofovir alafenamide

10.3.3. AD-120-2008: Metabolism of TAF in Dog

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340	Metabolism	[^{14}C]TAF	AD-120-2008

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	Male/10
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Not fasted
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample	Plasma
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

Final Metabolite Designation	Plasma Concentrations (ng Equivalents [^{14}C]GS-7340/g)						AUC_{0-24h} (ng eq•h/g)	% AUC of Total Radioactivity
	0.25 h	0.5 h	2 h	4 h	12 h	24 h		
M2	ND	45.7	58.5	69.1	ND	30.4	1212	7.61
M3	ND	49.0	85.2	67.3	ND	ND	265	1.67
M5	ND	ND	36.9	ND	ND	ND	36.9	0.23
M9	ND	ND	22.2	20.8	ND	ND	65.2	0.41
M10	ND	ND	23.7	ND	ND	ND	23.7	0.15
M12	2760	2070	1050	541	278	175	10874	68.3
M17	213	132	ND	ND	ND	ND	69.8	0.44
M18	4640	1840	38.4	ND	ND	ND	2799	17.6
M20	92.4	76.3	ND	ND	ND	ND	32.6	0.20
TAF	616	420	ND	ND	ND	ND	207	1.30

NA = not applicable; ND = peak was not detected or below the established limit of quantitation; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340	Metabolism	**[^{14}C]TAF**	AD-120-2008

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	Male/10
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Not fasted
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample	Urine
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

	Urine (Percent of Radioactive Dose)				
	Collection Interval (h)				
Final Metabolite Designation	**0-24**	**24-48**	**48-72**	**72-96**	**Total**
M1	ND	ND	ND	ND	ND
M2	0.88	0.28	0.16	0.19	1.49
M4	0.69	0.06	0.03	0.02	0.79
M5	0.47	ND	ND	ND	0.47
M11	0.43	ND	ND	ND	0.43
M12	13.3	5.08	3.43	2.35	24.2
M15	0.22	0.02	ND	ND	0.23
M18	ND	0.12	0.07	0.04	0.23
M23	ND	ND	ND	ND	ND
TAF	1.32	ND	ND	ND	1.32

TAF = tenofovir alafenamide; ND = peak was not detected or below the established limit of quantitation

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340	Metabolism	**[^{14}C]TAF**	AD-120-2008

Methods:

Species	Bile Duct-Cannulated Beagle dogs
Sex (M/F) / No. of Animals	Male/10
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Not fasted
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample	Urine
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

	Urine (Percent of Radioactive Dose)				
	Collection Interval (h)				
Final Metabolite Designation	**0-24**	**24-48**	**48-72**	**72-96**	**Total**
M1	0.12	0.04	0.04	ND	0.19
M2	1.06	0.35	0.14	0.15	1.70
M4	0.36	0.06	0.05	ND	0.48
M5	0.32	0.06	0.03	0.04	0.44
M11	0.39	0.04	ND	ND	0.42
M12	8.97	3.75	2.41	1.68	16.8
M15	ND	ND	ND	ND	ND
M18	ND	ND	ND	ND	ND
M23	0.24	ND	ND	ND	0.24
TAF	1.30	ND	ND	ND	1.30

TAF = tenofovir alafenamide; ND = peak was not detected or below the established limit of quantitation

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340	Metabolism	[^{14}C]TAF	AD-120-2008

Methods:

Species Bile Duct-Cannulated Beagle dogs
Sex (M/F) / No. of Animals Male/10
Method of Administration: Oral gavage
Dose (mg/kg): 15
Feeding Condition: Not fasted
Vehicle Formulation water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay: [^{14}C]TAF/Liquid Scintillation Counter

	Bile (Percent of Radioactive Dose)			
	Collection Interval (h)			
Final Metabolite Designation	0-2	2-4	4-6	Total
M2	0.08	0.02	0.02	0.12
M4	0.23	0.05	0.04	0.32
M5	0.23	0.10	0.10	0.43
M6	0.07	0.02	0.01	0.10
M11	0.04	0.02	0.03	0.09
M12	0.42	0.23	0.32	0.96
M14	0.10	0.06	0.06	0.22
M16	2.72	0.84	0.82	4.38
M17	0.29	0.09	0.10	0.48
M18	2.07	0.64	0.66	3.37
M19	0.01	0.04	0.04	0.09
M21	0.14	0.13	0.11	0.38
M22	0.07	0.09	0.07	0.23
M23	0.02	0.15	0.08	0.24
TAF	0.06	0.09	0.05	0.19

TAF = tenofovir alafenamide; ND = Peak was not detected or below the established limit of quantitation

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340	Metabolism	**[^{14}C]TAF**	AD-120-2008

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	Male/10
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Not fasted
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample	Feces
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

	Intact Dog (Feces, Percent of Radioactive Dose)			BDC Dog (Feces, Percent of Radioactive Dose)		
	Collection Interval (h)			Collection Interval (h)		
Final Metabolite Designation	**0-24**	**24-48**	**Total**	**0-24**	**24-48**	**Total**
M2	0.21	0.04	0.25	ND	ND	ND
M11	0.35	ND	0.35	0.50	0.20	0.70
M12	18.3	2.49	20.8	16.0	10.4	26.4

TAF = tenofovir alafenamide; ND = Peak not detected or below the established limit of quantitation; BDC = bile duct-cannulated

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340	Metabolism	**[^{14}C]TAF**	AD-120-2008

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	Male/10
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Not fasted
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample	Bone
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

	Single Dose		**Multiple Dose**	
	Bone Concentration (ng equivalents [^{14}C]GS-7340/g)			
	Collection Time (h)			
Final Metabolite Designation	**2**	**24**	**2**	**24**
M2	ND	ND	52.9	ND
M6	ND	ND	45.1	ND
M12	2190	1070	4000	2820

TAF = tenofovir alafenamide; ND = peak not detected or below the established limit of quantitation

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340	Metabolism	**[^{14}C]TAF**	AD-120-2008

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	Male/10
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Not fasted
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample	Liver
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

	Single Dose		**Multiple Dose**	
	Liver Concentration (ng equivalents [^{14}C]GS-7340/g)			
	Collection Time (h)			
Final Metabolite Designation	**2**	**24**	**2**	**24**
M2	3120	1230	4440	2610
M7	3840	1160	ND	3260
M8	7760	2680	11700	7340
M12	58200	43300	68100	49400
M13	ND	336	723	716
M18	1570	1040	556	294

TAF = tenofovir alafenamide; ND = peak not detected or below the established limit of quantitation

11. PHARMACOKINETICS: EXCRETION

11.1. FTC

Results from FTC excretion studies are described in Sections 5.1.1 10.1.1, 10.1.2, and 10.1.3 of this document.

11.2. RPV

Results from the TAF excretion studies are described in Sections 10.2.1, 10.2.2, 10.2.4 and 10.2.5 of this document.

11.3. TAF and TFV

Results from the TAF excretion studies are described in Sections 5.3.1, 5.3.2, and 12.1.1 of this document.

11.3.1. 96-DDM-1278-001: Effect of Dose on the Recovery of Radioactivity Following Administration of [^{14}C]TFV to Rats

Report Title	Study Type	Test Article	Report Number
Effect of Dose on Recovery of [^{14}C]PMPA Following Intravenous Administration to Sprague-Dawley Rats	Excretion	TFV or TDF	96-DDM-1278-001

Species	Rat
Sex (M/F) No. of Animals	6M (4M group 1, 2M group 2)
Feeding Condition	Fasted
Vehicle Formulation	Sterile saline or phosphate buffered saline
Method of Administration	IV single bolus
Dose (mg/kg/day)	10 (group 1), 50 (group 2)
Analyte (Radionuclide)	Total radioactivity, % recovery ^{14}C
Specific Activity	400 µCi/kg
Assay	Liquid scintillation counting

Excretion Route	**Urine (+ cage wash) (% dose in sample)**	**Feces (% dose in sample)**	**% Total recovery**	
Time			**Urine**	**Feces**
10 mg/kg				
0–24 h	85.2	3.18		
24–168 h	7.6	1.30	92.7	4.48
50 mg/kg				
0–24 h	77.5	7.39		
24–168 h	6.5	1.07	84.0	8.46

12. PHARMACOKINETICS: EXCRETION INTO BILE

12.1. TAF and TFV

12.1.1. AD-120-2007: Excretion of [^{14}C]TAF Following Single oral Administration in Dog

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Intact and Bile Duct-Cannulated Dogs	Excretion	[^{14}C]TAF	AD-120-2007

Methods:

Species	Beagle dogs
Sex (M/F)/No. of Animals	M/3
Method of Administration	Oral gavage
Dose (mg/kg)	15
Feeding Condition	Fasted
Specific Activity	10 µCi/mg
Radionuclide	Carbon-14
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

Tabulated PK Results :

Sample Type	Plasma	Blood
T_{max} (h)	0.25 ± 0.00	0.25 ± 0.00
C_{max} (ng eq/g)	8830 ± 774	7320 ± 895
t½ (h)	30.8 ± 4.34	107 ± 30.0
AUC_{0-t} (ng eq•h/g)	18500 ± 2020	44600 ± 11900

F = female; M = male; TAF = tenofovir alafenamide; NA = not applicable

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Intact and Bile Duct-Cannulated Dogs	Excretion	[^{14}C]TAF	AD-120-2007

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/3
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	10 µCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/ Liquid Scintillation Counter

	Percent of Radioactive Dose (%)			
Time Point	**Urine**	**Feces**	**Cage Rinse**	**Total**
0-8 h	10.2 ± 4.17	NA	NA	10.3 ± 4.17
0-24 h	18.6 ± 4.23	29.5 ± 7.03	2.46 ± 2.33	50.6 ± 6.40
0-48 h	24.5 ± 4.30	35.0 ± 2.58	3.69 ± 2.72	63.2 ± 2.78
0-72 h	28.4 ± 4.41	35.9 ± 2.55	4.63 ± 3.29	68.9 ± 2.21
0-96 h	31.1 ± 4.46	36.4 ± 2.46	5.28 ± 3.34	72.8 ± 2.09
0-120 h	33.1 ± 4.56	36.9 ± 2.34	5.82 ± 3.48	75.8 ± 1.85
0-144 h	34.6 ± 4.63	37.2 ± 2.29	6.26 ± 3.44	78.1 ± 1.97
0-168 h	35.9 ± 4.69	37.4 ± 2.21	NA	73.3 ± 5.27

F = female; M = male; TAF = tenofovir alafenamide; NA = not applicable

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Intact and Bile Duct-Cannulated Dogs	Excretion	[^{14}C]TAF	AD-120-2007

Methods:

Species	Bile Duct-Cannulated Beagle dogs
Sex (M/F) / No. of Animals	M/3
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	10 μCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/ Liquid Scintillation Counter

	Cumulative Excretion of Radioactivity (% of dose)				
Time Point	**Urine**	**Feces**	**Cage Rinse**	**Bile**	**Total**
0-1 h	NA	NA	NA	2.94 ± 2.56	2.94 ± 2.56
0-2 h	NA	NA	NA	6.80 ± 4.14	6.80 ± 4.13
0-4 h	1.72 ± 2.98	NA	NA	9.48 ± 2.24	11.2 ± 5.13
0-6 h	NA	NA	NA	12.2 ± 2.45	12.2 ± 2.43
0-8 h	8.25 ± 0.90	NA	NA	12.4 ± 2.53	20.6 ± 3.30
0-12 h	NA	NA	NA	12.5 ± 2.58	12.5 ± 2.58
0-24 h	13.9 ± 1.24	22.8 ± 19.6	0.77 ± 0.12	12.9 ± 2.70	50.3 ± 22.5
0-48 h	18.3 ± 1.50	40.8 ± 2.91	1.45 ± 0.38	13.3 ± 2.90	73.9 ± 0.90
0-72 h	21.0 ± 1.90	42.2 ± 3.61	1.81 ± 0.60	13.7 ± 3.04	78.7 ± 0.83
0-96 h	22.9 ± 1.84	42.4 ± 3.64	2.05 ± 0.63	13.8 ± 3.11	81.2 ± 0.49
0-120 h	24.5 ± 2.14	42.5 ± 3.58	2.25 ± 0.62	14.0 ± 3.16	83.3 ± 0.81
0-144 h	25.5 ± 2.29	42.6 ± 3.63	2.42 ± 0.62	14.0 ± 3.21	84.6 ± 1.02
0-168 h	26.5 ± 2.44	42.7 ± 3.64	NA	14.0 ± 3.21	83.3 ± 1.77

F = female; M = male; TAF = tenofovir alafenamide; NA = not applicable

12.1.2. 96-DDM-1278-002: A Study of Biliary Excretion of [^{14}C]TFV in the Dog

Report Title	Study Type	Test Article	Report Number
A Pilot Study of Biliary Excretion of [^{14}C]PMPA in the Beagle Dog	Excretion	TFV or TDF	96-DDM-1278-002

Species	Dog
Sex (M/F) No. of Animals	1M
Feeding Condition	Fasted
Vehicle Formulation	aqueous solution
Method of Administration	IV Bolus, single administration
Dose (mg/kg)	10
Radionuclide	^{14}C
Specific Activity	5 μCi/mg
Assay	Liquid scintillation counting

Excretion Route	Urine (% dose)	Feces (% dose)	Bile (% dose)	Cage wash (% dose)	Total (% dose)
Time					
0–48 h	70.0	0.42	0.26	5.68	76.4

F = female; M = male; TAF = tenofovir alafenamide; NA = not applicable

13. PHARMACOKINETICS: INDUCTION/INHIBITION OF DRUG METABOLIZING ENZYMES

13.1. FTC

13.1.1. 15247: Evaluation of FTC as an Inhibitor of Human Cytochromes P450 and Uridine Diphosphate Glucuronosyl Transferase Activity

Report Title	Study Type	Test Article	Report Number
In Vitro Evaluation of Emtricitabine (FTC) as an Inhibitor of Human Cytochrome P450 Enzymes and 5'-Uridine Diphosphate Glucuronosyl Transferase (UGT)	Metabolism	FTC	15247

Methods: Pooled human hepatic microsomal fraction was utilized to measure the activities: 7-ethoxyresorufin *O*-deethylation (CYP1A2), coumarin 7-hydroxylation (CYP2A6), 7-benzyloxyresorufin *O*-debenzylation (CYP2B6), tolbutamide methyl hydroxylation (CYP2C9), (*S)* mephenytoin 4'-hydroxylation (CYP2C19), dextromethorphan *O*-demethylation (CYP2D6), chlorzoxazone 6-hydroxylation (CYP2E1), testosterone 6β-hydroxylation (CYP3A) and 7-hydroxycoumarin glucuronidation (various UGTs), in the presence of various concentrations of FTC. Selective inhibitors were tested to confirm the sensitivity of the assays

Enzyme	Enzyme Reaction	Control Inhibitor	FTC		Control Inhibitor
			K_i (μM)	Type of Inhibition	K_i (μM)
CYP1A2	7-ethoxyresorufin *O*-deethylation	α-naphthoflavone	-	No inhibition	Competitive 0.011
CYP2A6	coumarin 7-hydroxylation	tranylcypromine	-	No inhibition	Mixed: Competitive 1.2/Uncompetitive 0.72
CYP2B6	7-benzyloxyresorufin *O*-debenzylation	orphenadrine	-	No inhibition	Competitive 200
CYP2C9	tolbutamide methyl-hydroxylation	sulfaphenazole	-	No inhibition	Mixed: Competitive 24/Uncompetitive 61
CYP2C19	*(S)* mephenytoin 4'-hydroxylation	ticlopidine	-	No inhibition	Complete inhibition
CYP2D6	dextromethorphan *O*-demethylation	quinidine	-	No inhibition	Competitive 0.046
CYP2E1	chlorzoxazone 6-hydroxylation	4-methylpyrazole	1788	Competitive inhibition	Mixed: Competitive 16/Uncompetitive 7.4
CYP3A	testosterone 6β-hydroxylation	ketoconazole	-	No inhibition	Competitive 0.044
UGT	7-hydroxycoumarin glucuronidation	-	-	No inhibition	-

Conclusion:

- FTC was not an inhibitor for human CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1 and 3A
- FTC did not show inhibition of 7-hydroxycoumarin glucuronidation

13.1.2. AD-162-2005: In Vitro Assessment of Induction Potential of Metabolizing Enzymes

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of Induction Potential of GS-9019 in Humans	Metabolism	FTC	AD-162-2005

Methods: The potential for induction of human drug metabolizing enzymes and transporters through the activation of the aryl hydrocarbon receptor (AhR) and the pregnane X receptor (PXR) by GS-9019 was assessed in vitro. Assessments of induction were done using Puracyp's hepatoma-derived cell lines, DRE12.6 and DPX2. DPX2 cells are stably transformed with an expression vector for human PXR and a reporter gene vector containing the enhancer regions of CYP3A4 linked to luciferase. DRE12.6 cells are transformed with an expression vector for human AhR and the Drug/Dioxin Response Element (DRE) of the human CYP1A2 gene linked to a luciferase reporter. Following 24 h of exposure to the test articles, medium was replaced with phosphate-buffered saline and MultiTox-Fluor™ Multiplex assay buffer at 1:1 ratio. The plates were incubated for a further 1 hr and fluorescence determined in a Perkin-Elmer Victor 2 fluorometer (excitation 400 nm, emission 510 nm). Triplicate values were averaged and the fold induction determined by comparison with the appropriate DMSO vehicle control concentration.

	Human PXR Activation		Human AhR Activation	
	Fold Induction Over 0.1% DMSO Control		Fold Induction Over 0.1% DMSO Control	
Concentration (µM)	FTC	Rifampicin	FTC	β-Naphthoflavone
0.1	–	3.02	–	1.73
0.15	0.84	–	1.00	–
0.5	0.88	4.57	0.96	3.69
1.0	–	6.30	–	5.54
1.5	0.84	–	0.91	–
5	0.83	13.21	0.88	25.04
10	–	13.80	–	35.69
15	1.22	–	0.91	–
20	–	14.58	–	50.07
50	1.44	–	1.14	–

13.2. RPV

13.2.1. TMC278-NC192: Ex Vivo Assessment of Induction and/or Inhibition of Drug Metabolizing Enzymes in Mouse

Report Title	Study Type	Test Article	Report Number
A Study of the Effects of TMC278 HCl on Some Hepatic Enzyme Activities After Oral Administration for 3 Months at Doses of 0, 20, 80, and 320 mg/kg/day to Make an Female Swiss Albino CD1 Mice	Metabolism	RPV	TMC278–NC192

Methods Microsomal fractions of livers from TMC278 HCl treated animals were isolated. Swiss albino CD1 mice were treated with 0 (0.5% (w/v) HPMC, vehicle), 20, 80 and 320 mg.base eq./kg/day TMC278 for three months. Liver microsomal fractions were analyzed for protein and total cytochrome P450 (CYP) content and for the enzyme activities shown below. Liver cytosolic fractions were analyzed for protein and GSH *S*-transferase activity towards CDNB as substrate. Results are presented for groups of 5 mouse liver pools, each pool being prepared from the livers of 2 mice.

	Control (vehicle only)		TMC278 (mg.base.eq/kg/day)					
			20		80		320	
Gender (M/F)	M	F	M	F	M	F	M	F
Microsomal protein[a]	17	16	18	16	19*	19**	25***	23***
CYP content[b]	1.2	0.83	1.2	0.99*	1.4*	1.0**	1.6***	1.2***
7-Ethoxyresorufin O-deethylase (CYP1A)[c]	315	223	378	284	362	322**	273	187
7-Pentoxyresorufin O-dependentylase (CYP2B)[c]	86	150	103	168	92	191**	88	135
4-Nitrophenol hydroxylase (CYP2E)[d]	1.9	2.4	2.1	2.3	2.3**	2.6	1.8	2.1
Testosterone 6β-hydroxylase (CYP3A)[d]	2.7	2.1	3.0	3.2**	4.2***	3.6***	4.7***	3.6***
Lauric acid 11-hydroxylase[d]	1.1	1.2	1.1	1.4	1.4	2.6***	3.4***	4.3***
Lauric acid 12-hydroxylase (CYP4A)[d]	0.83	1.5	1.2*	1.9	4.4***	7.8***	21***	30***
UDPglucuronosyltransferase (substrate thyroxine)[c]	16	11	17	16*	23**	19***	33***	26***
Cytosolic protein[a]	97	106	93	102	97	108	115**	133***
GSH *S*-transferase (substrate CDNB)[e]	8.1	2.0	8.1	2.3	6.3*	2.2	3.6***	1.7

Additional Information
Values significantly different from control are: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$
a Units : mg protein/g liver.
b Units : nmol/mg protein.
c Units : pmol/min/mg protein.
d Units : nmol/min/mg protein.
e Units : µmol/min/mg protein
CNDB: 1-chloro2,4-dinitrobenzene; CYP: cytochrome 450; GSH: glutathione; UDP: uridine diphosphate; HPMC: hydroxypropyl methylcellulose

13.2.2. TMC278-NC193: Ex Vivo Assessment of Induction and/or Inhibition of Drug Metabolizing Enzymes in Rat

Report Title	Study Type	Test Article	Report Number
A Study of the Effects of TMC278 on Some Hepatic Enzyme Activities After Oral Administration For 6 Months at Doses of 0, 40, 120, and 400 mg/kg/day to Male and Female Sprague-Dawley Rats	Metabolism	RPV	TMC278–NC193

Methods Microsomal fractions of livers from TMC278 base treated animals were isolated. Sprague Dawley rats were treated with 0 (100 mg/ml citric acid in PEG400, vehicle), 40, 120 and 400 mg/kg/day TMC278 for 6 months. Liver microsomal fractions were analyzed for protein and GSH *S*-transferase activity towards CDNB as substrate. Results are presented for groups of 5 rats.

	Control (vehicle only)		TMC278 (mg/kg/day)					
			40		120		400	
Gender (M/F)	M	F	M	F	M	F	M	F
Microsomal protein[a]	61	45	65	45	66	47	72**	54***
CYP content[b]	0.84	0.56	0.83	0.56	0.81	0.63*	0.87	0.69***
7-Ethoxyresorufin O-deethylase (CYP1A)[c]	28	24	24	26	21	32*	18*	29
7-Pentoxyresorufin O-dependentylase (CYP2B)[c]	50	11	43	11	52	14*	37	13*
4-Nitrophenol hydroxylase (CYP2E)[d]	0.64	0.73	0.62	0.69	0.60	0.77	0.46*	0.69
Testosterone 6β-hydroxylase (CYP3A)[d]	0.64	0.05	0.61	0.06	0.80	0.15***	0.77	0.30***
Lauric acid 11-hydroxylase[d]	0.42	0.34	0.47	0.30	0.57*	0.37	0.77***	0.42*
Lauric acid 12-hydroxylase (CYP4A)[d]	0.47	0.44	0.66	0.33	1.2**	0.41	2.2***	0.56*
UDPglucuronosyltransferase (substrate thyroxine)[c]	5.7	5.6	3.7**	7.1	4.4*	5.5	7.1*	7.5
Cytosolic protein[a]	130	12	132	112***	132	111***	134	117*
GSH *S*-transferase (substrate CDNB)[e]	2.6	1.2	2.5	1.7*	2.4	1.6	2.3	1.9**

Additional Information
Values significantly different from control are: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$
a Units : mg protein/g liver. b Units : nmol/mg protein. c Units : pmol/min/mg protein. d Units : nmol/min/mg protein. e Units : µmol/min/mg protein
CNDB: 1-chloro2,4-dinitrobenzene; CYP: cytochrome 450; GSH: glutathione; UDP: uridine diphosphate; HPMC: hydroxypropyl methylcellulose

13.2.3. TMC278-NC140: Ex Vivo Assessment of Induction and/or Inhibition of Drug Metabolizing Enzymes in Dog

Report Title	Study Type	Test Article	Report Number
A Study of the Effects of TMC278 on Some Hepatic Enzyme Activities After Oral Administration for 6 Months at Doses of 0, 5, 10, and 40 mg/kg/day to Male and Female Beagle Dogs	Metabolism	RPV	TMC278–NC140

Methods Microsomal and cytosolic fractions of livers from TMC278 base treated animals were isolated. Male and female Beagle dogs were treated with 0 (citric acid (100 mg/ml) in PEG400, vehicle solution) 5, 10 and 40 mg/kg/day TMC278 for six months. Liver microsomes were analysed for protein and total cytochrome P450 (CYP) content and for the enzyme activities shown below. Cytosolic fractions were analyzed for protein content and GSH *S*-transferase activity. Groups of 2 male and 2 female control and TMC278 treated beagle dogs were combined for statistical analysis of data.

		TMC278 (mg/kg/day)		
	Control (vehicle only)	5	10	40
Microsomal protein[a]	40.7	42.3	44.4	43.2
CYP content[b]	0.72	0.72	0.75	0.70
7-Ethoxyresorufin O-deethylase (CYP1A)[c]	183	169	217	162
7-Pentoxyresorufin O-dependentylase (CYP2B)[c]	76	84	96	87
4-Nitrophenol hydroxylase (CYP2E)[d]	0.50	0.59	0.53	0.51
Testosterone 6β-hydroxylase (CYP3A)[d]	0.58	0.49	0.33**	0.43*
Lauric acid 11-hydroxylase[d]	0.21	0.22	0.22	0.22
Lauric acid 12-hydroxylase (CYP4A)[d]	0.83	0.83	0.85	0.94
UDPglucuronosyltransferase (substrate thyroxine)[c]	2.8	2.3	2.1	1.9
Cytosolic protein[a]	128	129	124	124
GSH *S*-transferase (substrate CDNB)[e]	1.24	1.02	1.04	1.00

Additional Information
Values significantly different from control are: * $p < 0.05$; ** $p < 0.01$
a units: mg protein/g liver; [b] units: nmol/mg protein; [c] units: pmol/min/mg protein; [d] units: nmol/min/mg protein; [e] units: µmol/min/mg protein
CNDB: 1-chloro2,4-dinitrobenzene; CYP: cytochrome 450; GSH: glutathione; UDP: uridine diphosphate; PEG400: polyethylene glycol 400

13.2.4. TMC278-NC186: In Vitro Assessment of Induction Potential of Drug Metabolizing Enzmes

Report Title	Study Type	Test Article	Report Number
An In Vitro Study to Asess the Potential of TMC278 to Induce CYP Enzyme Activities in Cryopreserved Human Hepatocytes	Metabolism	RPV	TMC278–NC186

Methods After establishment of the hepatocyte cultures, human hepatocytes were treated either with vehicle (DMSO), with various concentrations of TMC278 or with the positive control compounds, omeprazole, rifampicin, or ethanol for 48 h. At the end of the treatment period, induction of CYP activities (CYP1A2, CYP2B6, CYP2C19, CYP2E1, CYP3A4) was measured based on the probe substrate metabolism. Mean fold induction of the different CYP-isoforms in cryopreserved human hepatocytes treated with TMC278 and positive controls was expressed against the vehicle control. In addition, induction of CYP activities was also determined by measurement of mRNA expression levels by TaqMan real-time RT-PCR. In total, three different individual batches of cryopreserved human hepatocytes were used in this study. The results are tabulated in the table below and each value is mean of three observations.

	Mean fold induction in enzyme activity levels				
	CYP1A2	**CYP2B6**	**CYP2C19**	**CYP2E1**	**CYP3A4**
Control (Vehicle)	1.00	1.00	1.00	1.00	1.00
TMC278 (2.5 µM)	1.06	1.28	1.44	0.98	0.24
TMC278 (10 µM)	0.62	0.71	1.20	1.37	0.04
TMC278 (25 µM)	0.51	0.58	1.25	0.93	0.05
Rifampicin (50 µM)	NA	2.60	3.07	NA	14.43
Rifampicin (50 µM) + TMC278 (25 µM) [a]	NA	0.43	0.28	NA	0.10
Omeprazole (25 µM)	4.95	NA	NA	NA	NA
Omeprazole (25 µM) + TMC278 (25 µM) [a]	2.35	NA	NA	NA	NA
Ethanol (100 mM)	NA	NA	NA	1.24	NA
Ethanol (100 mM) + TMC278 (25 µM) [a]	NA	NA	NA	0.76	NA

Additional Information
a Inhibition control to investigate interference of TMC278 with measurement of CYP activities;
CYP: cytochrome 450; mRNA: messenger ribonucleic acid; NA: not applicable; RT-PCR: reverse transcriptase-polymerase chain reaction; DMSO: dimethylsulfoxide

Report Title	Study Type	Test Article	Report Number
An In Vitro Study to Asess the Potential of TMC278 to Induce CYP Enzyme Activities in Cryopreserved Human Hepatocytes	Metabolism	RPV	TMC278–NC186

	Mean fold change in mRNA expression levels				
	CYP1A2	**CYP2B6**	**CYP2C19**	**CYP2E1**	**CYP3A4**
Control (Vehicle)	1.00	1.00	1.00	1.00	1.00
TMC278 (2.5 µM)	2.55	2.89	1.19	0.81	27.12
TMC278 (10 µM)	3.17	2.96	1.12	0. 56	25.95
TMC278 (25 µM)	3.58	1.18	0.60	1.12	5.08
Rifampicin (50 µM)	NA	6.80	1.69	NA	54.88
Omeprazole (25 µM)	15.07	NA	NA	NA	NA
Ethanol (100 mM)	NA	NA	NA	1.01	NA

13.3. TAF and TFV

13.3.1. AD-120-2003: Human Cytochrome P450 Inhibition Potential of TAF

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of Human Liver Cytochrome P450 Inhibition Potential of GS-7340	Metabolism	TAF	AD-120-2003

Methods The inhibitory effect of TAF on human P450 enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) was investigated using human liver microsomes in the presence of NADPH at concentrations of TAF up to 25 µM

Enzyme	Activity	Calculated IC_{50} (µg/mL)	
		TAF	Control Inhibitor[a]
CYP1A2	Phenacetin O-deethylase	>25	0.05
CYP2B6	Bupropion 4-hydroxylase	>25	0.72
CYP2C8	Paclitaxel 6α-hydroxylase	>25	0.34
CYP2C9	Tolbutamide 4-hydroxylase	>25	0.64
CYP2C19	(S) Mephenytoin 4'-hydroxylase	>25	7.65
CYP2D6	Dextromethorphan O-demethylase	>25	0.05
CYP3A	Midazolam 1'-hydroxylase	7.6	0.03
	Testosterone 6β-hydroxylase	7.4	0.11

CYP = cytochrome P450 enzyme; TAF = tenofovir alafenamide

a Control inhibitors: CYP1A2, α Naphthoflavone (0-3 µM); CYP2B6, Ticlopidine (0-10 µM); CYP2C8, Montelukast (0-3 µM); CYP2C9, Sulfaphenazole (0-10 µM); CYP2C19, Tranylcypromine (0-50 µM); CYP2D6, Quinidine (0-3 µM); CYP3A, Ketoconazole (0-3 µM)

13.3.2. V990172-104: Human Cytochrome P450 Inhibition Potential of TFV

Report Title	Study Type	Test Article	Report Number
The Effect of TFV and TDF on the Activities of the Cytochrome P-450 Isoforms in Human Hepatic microsomes	Metabolism	TFV	V990172-104

Method Probe activities selective for the CYP enzymes were utilized to examine the potential inhibitory effects of TFV using human hepatic microsomal fraction as the catalyst. Activities were evaluated in the presence and absence of 100 μM TFV.

CYP Enzyme (activity)	Control (nmol/mg/min)	TFV (nmol/mg/min)
CYP3A (terfenadine hydroxylation)	0.018 ± 0.009	0.016 ± 0.009
CYP2D6 (dextromethorphan O-demethylation)	0.066 ± 0.041	0.064 ± 0.043
CYP2C9 (tolbutamide 4-hydroxylation)	0.218 ± 0.093	0.216 ± 0.096
CYP2E1 (chlorzoxazone 6-hydroxylation)	1.48 ± 0.58	1.50 ± 0.65
CYP1A2 (7-ethoxycoumarin O-deethylation)	0.481 ± 0.182	0.487 ± 0.19

CYP = cytochrome P450 enzyme; TFV = tenofovir
a Significant compared to control, $p < 0.05$; n = 3–4

13.3.3. AD-120-2040: Human CYP Mechanism-Based Inhibition of TAF

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of Human Hepatic Microsomal Cytochrome P450 Mechanism Based Inhibition Potential of GS 7340	Metabolism	TAF	AD-120-2040

Methods The potential for test compound to act as a mechanism based inhibitor of human hepatic microsomal cytochromes P450 drug metabolizing enzymes, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6, was assessed. Two-stage incubation protocol was used, with the first stage allowing inactivation of the enzyme in the absence of substrate, and the second stage being used to assay remaining enzyme activity. A 10-fold dilution was performed between the 2 stages to reduce the direct inhibitory effects of the test compounds. The enzyme-specific metabolites were quantified by LC MS/MS, except for resorufin (CYP1A2 metabolite), which was quantified fluorometrically.

Test Compound	Probe Activity	Calculated % Change	
		Control Inhibitor[a]	TAF
CYP1A2	Ethoxyresorufin O-deethylase	71.2 ± 1.9	-5.61 ± 9.16
		57.9 ± 3.1	
CYP2B6	Bupropion 4-hydroxylase	85.4 ± 0.8	-5.29 ± 3.52
CYP2C8	Paclitaxel 6α-hydroxylase	60.3 ± 4.8	17.4 ± 14.2
CYP2C9	Diclofenac 4'-hydroxylase	79.6 ± 2.5	-7.36 ± 17.2
CYP2C19	S-Mephenytoin 4'-hydroxylase	62.3 ± 0.9	5.30 ± 4.10
CYP2D6	Dextromethorphan O-demethylase	80.1 ± 3.6	3.94 ± 16.1
CYP3A	Midazolam 1'-hydroxylase	60.1 ± 1.8	16.4 ± 5.87
		80.2 ± 1.2	
	Testosterone 6β-hydroxylase	91.3 ± 0.5	10.3 ± 4.66
		70.7 ± 1.2	

CYP = cytochrome P450 enzyme; TAF = tenofovir alafenamide
a CYP1A2, furafylline and resveratrol; CYP2B6, ticlopidine; CYP2C8, gemfibrozil glucuronide; CYP2C9, tienilic acid; CYP2C19, ticlopidine; CYP2D6, paroxetine; CYP3A, mibefradril and mifepristone

13.3.4. AD-120-2005: Induction of Metabolizing Enzymes by TAF In Vitro

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of Induction Potential of GS-7340 in Humans	Metabolism	TAF	AD-120-2005

Methods Assessments of induction were done using Puracyp's hepatoma-derived cell line DPX2. DPX2 cells are stably transformed with an expression vector for human PXR and a reporter gene vector containing the enhancer regions of CYP3A4 linked to luciferase. Following 24 hours of exposure to the test articles, the luciferase substrate was added and the luminescence was read in a luminometer. The average luminescent units for the 3 replicates were divided by the average for the DMSO solvent control to determine the fold induction

	Fold Induction Over 0.1% DMSO Control (PXR Activation)	
Concentration	TAF	Rifampicin
0.1 µM	NA	1.56
0.15 µM	0.81	NA
0.5 µM	0.92	4.59
1.0 µM	NA	6.36
1.5 µM	0.88	NA
5 µM	1.03	12.5
10 µM	NA	13.4
15 µM	1.58	NA
20 µM	NA	12.6
50 µM	3.89	NA

TAF = tenofovir alafenamide; NA = not applicable

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of Induction Potential of GS-7340 in Humans	Metabolism	TAF	AD-120-2005

Test Compound	Fold Induction Over 0.1% DMSO Control (AhR Activation)	
	TAF	β-Naphthoflavone
0.1 µM	NA	2.56
0.15 µM	1.09	NA
0.5 µM	1.04	6.37
1.0 µM	NA	11.1
1.5 µM	0.97	NA
5 µM	0.91	47.5
10 µM	NA	40.0
15 µM	0.87	NA
20 µM	NA	27.7
50 µM	0.90	NA

TAF = tenofovir alafenamide; NA = not applicable

13.3.5. AD-120-2032: Assessment of Induction Potential of TAF in Human Hepatocyte In Vitro

Report Title	Study Type	Test Article	Report Number
Evaluation of Induction Potential of GS-7340 in Cultured Human Hepatocytes	Drug-drug interaction (in vitro)	TAF	AD-120-2032

Methods The induction potential of cytochrome P450 (CYP) isoforms, P-glycoprotein (P-gp), and UDP glucuronosyl transferase (UGT) 1A1 by TAF was assessed in cultured human hepatocytes. GS-7340 was incubated in 3 preparations of cryopreserved human hepatocyte cultures at concentrations of 1, 10, and 100 µM with vehicle control and appropriate positive controls. Following 3 days of exposure, induction was determined in *situ* by catalytic activity assays, and mRNA expression was determined using real-time PCR (RT-PCR). Additionally, the cytotoxic potential of GS-7340 was assessed using the MTT assay.

Test Compound Concentration (µM)	Fold Induction							
	mRNA Expression in Human Hepatocytes					Human Hepatocytes		
	CYP1A2	CYP2B6	CYP3A4	P-gp	UGT1A1	CYP1A2	CYP2B6	CYP3A
1	1.2 ± 0.14	0.95 ± 0.17	0.92 ± 0.098	1.0 ± 0.037	0.94 ± 0.074	1.0 ± 0.20	1.1 ± 0.047	0.97 ± 0.083
10	3.0 ± 0.47	1.6 ± 0.26	8.3 ± 1.4	1.1 ± 0.11	1.7 ± 0.15	1.4 ± 0.15	0.85 ± 0.085	0.99 ± 0.057
100	6.9 ± 0.86	2.5 ± 0.21	44 ± 3.6	0.87 ± 0.093	3.9 ± 0.47	0.84 ± 0.065	0.42 ± 0.095	0.37 ± 0.025

TAF = tenofovir alafenamide
Note: Positive control for CYP1A2 was omeprazole, for CYP2B6 and P-gp was phenobarbital, for CYP3A was rifampicin, and for UGT1A1 was β-naphthoflavone.

Report Title	Study Type	Test Article	Report Number
Evaluation of Induction Potential of GS-7340 in Cultured Human hepatocytes	Drug-drug interaction (in vitro)	TAF	AD-120-2032

Methods The induction potential of cytochrome P450 (CYP) isoforms, P-glycoprotein (P-gp), and UDP glucuronosyl transferase (UGT) 1A1 by TAF was assessed in cultured human hepatocytes. GS-7340 was incubated in 3 preparations of cryopreserved human hepatocyte cultures at concentrations of 1, 10, and 100 µM with vehicle control and appropriate positive controls. Following 3 days of exposure, induction was determined in *situ* by catalytic activity assays, and mRNA expression was determined using real-time PCR (RT-PCR). Additionally, the cytotoxic potential of GS-7340 was assessed using the MTT assay.

Test Compound Concentration (µM)	Cell Viability (% Control Incubations)[a]			
	Hepatocyte Lot 228	Hepatocyte Lot 307	Hepatocyte Lot 321	Mean
0	100 ± 6	100 ± 14	100 ± 7	100 ± 9
1	104 ± 3	120 ± 10	104 ± 6	109 ± 6
10	58 ± 2	90 ± 13	81 ± 2	76 ± 6
100	47 ± 1	73 ± 8	54 ± 2	58 ± 4

TAF = tenofovir alafenamide
a Values are the mean ± standard deviation of triplicate determinations and represent percentage viability relative to solvent vehicle-only treated cells

13.3.6. AD-120-2006: In Vitro Assessment of Human UGT1A1 Inhibition Potential of TAF

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of Human UGT1A1 Inhibition Potential of GS-7340	Metabolism	TAF	AD-120-2006

Methods The potential for TAF to inhibit the catalytic activity of human UGT1A1 was assessed. The rates of formation of β-estradiol-3-glucuronide from estradiol substrate by hepatic microsomal fractions were determined in the presence and absence of TAF, and, where possible, IC_{50} values were determined. Silybin was used as positive control.

Enzyme	Activity	Calculated IC_{50} (µM)	
		TAF	Silybin
UGT1A1	β-estradiol-3-glucuronidation	>50	1.69

TAF = tenofovir alafenamide; UGT = uridine diphosphate glucuronosyl transferase

14. PHARMACOKINETICS: DRUG-DRUG INTERACTIONS

14.1. FTC

Results from FTC transporter related drug-drug interaction studies are described as a component of Strivild® (STB) (elvitegravir (EVG)/cobicistat (COBI)/ /FTC/TFV) in Section 14.4.

14.2. RPV

14.2.1. TMC–NC194: Inhibition of Metabolism of Interacting Drugs by RPV

Report Title	Study Type	Test Article	Report Number
Inhibition of Metabolism of Interacting Drugs by TMC278	Metabolism	RPV	TMC278–NC194

Methods: The interaction of TMC278 with the metabolism of interacting drugs was investigated in a pooled batch of human liver microsomes. The inhibitory potential of TMC278 on the overall metabolism and/or the formation of their major metabolites is shown. The IC_{50}-values represent the concentration in µM or µg-base-eq/mL of TMC278 inhibiting the metabolism by 50%.

	IC_{50} (95% confidence interval)		Positive Control		
Concentration (µM)	**µM**	**µg-base-eq/mL**	**µM**	**Inhibitor**	**% Inhibition**
S-mephenytoin[a]	1.3[a] (0.74 ─ 1.8)	0.46[a] (0.27 ─ 0.65)	1	**3-benzyl-phenobarbital**	81
Sildenafil	1.4 (-0.13 ─ 3.0)	0.53 (-0.047 ─ 1.1)	1	**ketoconazole**	125
Clarithromycin	2.0 (0.042 ─ 4.0)	0.74 (0.015 ─ 1.46)	1	**ketoconazole**	93
Norethindron	3.9 (2.6 ─ 5.3)	1.44 (0.93 ─ 1.95)	1	**ketoconazole**	84
Sertraline	5.2 (-3.1 ─ 14)	1.9 (-1.1 ─ 4.9)	10[3]	**1-aminobenzotriazole**	167[b]
Paroxetine	6.6 (-1.2 ─ 14)	2.4 (-0.42 ─ 5.3)	3	**quinidine**	91
17α-Ethinyloestradiol[c]	6.5[c] (4.2 ─ 8.7)	2.4[c] (1.5 ─ 3.2)	1	**ketoconazole**	56/59[d]
Omeprazole	12.0 (7.0 ─ 17)	4.4 (2.6 ─ 6.2)	1/1	**3-benzyl-phenobarbital / ketoconazole**	92
Abacavir[e,f]	>30[f]	>11[f]	600	**4-methylpyrazole**	95
Chlorzoxazone[g]	>30[g]	>11[g]	100	**diethyldithiocarbamate**	-184[h]

Additional Information
a) As determined by the formation of the 4-hydroxy metabolite only.
b) This inhibition is not significantly different from the boiled fraction.
c) As determined by the formation of a hydroxy metabolite.
d) 56 / 59 % inhibition of metabolism of unchanged drug and inhibition of formation of a hydroxy metabolite, respectively.
e) Tested in cytosol fractions, not in microsomes.
f) As determined by disappearance from the unchanged abacavir, as well as the formation of its carboxylic acid metabolite.
g) As determined by disappearance from the unchanged chlorzoxazone, as well as the formation of its 6-hydroxy metabolite.
h) No inhibition was observed.

14.2.2. TMC278-FK10042: The possible inhibition by TMC278 in CHO cell lines overexpressing OCT2 (SLC22A2)

Report Title	Study Type	Test Article	Report Number
The possible inhibition by TMC278 in CHO cell lines overexpressing OCT2 (SLC22A2)	Drug-drug interaction (in vitro)	RPV	TMC278-FK10042

Methods: The aim of this study was to test whether TMC278 can inhibit OCT2(SLC22A2)-mediated transport. Using CHO-cells, stably transfected with cDNA encoding for this transporter, transport of a prototypical substrate of OCT2, metformin (10 µM, 1 min) was determined in the presence and absence of various concentrations of TMC278, quinidine and cimetidine. Transport, and inhibition thereof, was tested in the presence of 1% BSA.

Substrate	Inhibitor	Protein	IC_{50} (µM)	Max.inhibition (%)	at conc. (µM)
Metformin	TMC278	1% BSA	5.46 ± 0.50	90.6 ± 0.6	50
Metformin	Quinidine	1% BSA	13.3 ± 1.1	101 ± 0.3	1000
Metformin	Cimetidine	1% BSA	45.5 ± 4.8	96.9 ± 0.3	1000

CHO = Chinese hamster ovary; OCT = organic cation transporter; BSA = bovine serum albumin

14.2.3. TMC-278-FK10420: An in vitro study on the inhibition of MATE-1 (SLC47A1) and MATE-2K (SLC47A2) by RPV

Report Title	Study Type	Test Article	Report Number
An in vitro study on the inhibition of MATE-1 (SLC47A1) and MATE-2K (SLC47A2) by TMC278	Drug-drug interaction (in vitro)	RPV	TMC278-FK10420

Methods: The aim of this study was to test whether TMC278 inhibits MATE-1/-2K –mediated transport of ^{14}C-Tetra Ethyl Ammonium (TEA, MATE-1 and MATE-2K substrate) in CHO cells stably transfected with cDNA encoding for this transporter. The intracellular TEA was quantified by a liquid scintillation counter.

Test compound	Rate (pmol/mg protein/min)		Maximal activity (%)	Rate (pmol/mg protein/min)		Maximal activity (%)
	CHO-Parent	CHO-MATE-1		CHO-Parent	CHO-MATE-2K	
TEA	1.85 ± 0.12	43.7 ± 2.72	100	1.31 ± 0.53	7.47 ± 0.34	100
TEA + 0.05 µM TMC278	2.58 ± 1.26	50.1 ± 4.98	114	1.83 ± 0.18	3.98 ± 0.91	35.0
TEA + 0.3 µM TMC278	3.51 ± 1.89	38.9 ± 3.19	84.7	1.31 ± 0.53	3.61 ± 0.51	37.4
TEA + 1.5 µM TMC278	2.20 ± 0.43	40.4 ± 5.38	89.5	1.38 ± 0.35	3.35 ± 0.73	32.1
TEA + 10 µM TMC278	1.91 ± 0.29	19.7 ± 1.26	42.4	1.13 ± 0.44	2.75 ± 0.43	26.4
TEA + 50 µM TMC278	1.91 ± 0.71	6.60 ± 2.13	11.2	1.72 ± 0.17	1.91 ± 0.05	3.1
TEA + 1 µM Quinidine	2.09 ± 0.57	34.8 ± 3.73	78.3	NA	NA	NA
TEA + 50 µM Quinidine	2.69 ± 0.58	10.1 ± 2.45	17.6	NA	NA	NA
TEA + 0.03 µM Pyrimethamine	NA	NA	NA	1.22 ± 0.1	3.50 ± 0.51	37.0
TEA + 3 µM Pyrimethamine	NA	NA	NA	1.34 ± 0.39	2.44 ± 0.40	17.8

CHO = Chinese hamster ovary; MATE = multidrug and toxin extrusion protein; NA = not applicable

14.3. TAF and TFV

Results from some of the TFV transporter studies are described as a component of STB (EVG/COBI/FTC/TFV) in Section 14.4.

14.3.1. AD-120-2018: Bidirectional Permeability of TAF Through Monolayers of P-glycoprotein and BCRP Overexpressing Cells

Report Title	Study Type	Test Article	Report Number
Bidirectional Permeability of GS-7340 Through Monolayers of P-glycoprotein and BCRP Over-expressing Cells	Drug-drug interaction (in vitro)	TAF	AD-120-2018

Methods The potential for TAF to act as a substrate for P-gp (MDR1) and BCRP was tested in monolayers of either wild type, MDR1-transfected or BCRP-transfected Madin-Darby canine kidney (MDCK II) cells (MDCK II-WT, MDCK II-MDR1 and MDCK II-BCRP, respectively). The effects of transporter-selective inhibitors were also assessed.

Wild Type and MDR1 Transfected MDCKII Cells

Cell Type	Direction	Initial Conc. (µM)	Recovery (%)	P_{app} (x 10^{-6} cm/s)			Efflux Ratio
				R1	R2	Average	
MDCK II-WT	Cell-Free	7.9	112	45.7	NA	**45.7**	NA
	Forward	7.9	103	1.4	1.5	**1.5**	4.8
	Reverse	7.6	99	6.7	7.4	**7.1**	
MDCK II-MDR1	Forward	7.4	92	0.1	0.3	**0.2**	66.2
	Reverse	7.7	96	12.8	11.3	**12.1**	
MDCK II-MDR1 (10 µM Cyclosporin A)	Forward	9.4	91	1.3	1.2	**1.3**	5.6
	Reverse	8.4	90	6.8	7.4	**7.1**	

TAF = tenofovir alafenamide; MDR1 = P-glycoprotein (P-gp, ABCB1); NA = not applicable

Report Title	Study Type	Test Article	Report Number
Bidirectional Permeability of GS-7340 Through Monolayers of P-glycoprotein and BCRP Over-expressing Cells	Drug-drug interaction (in vitro)	TAF	AD-120-2018

Wild Type and BCRP Transfected MDCK II Cells

Cell Type	Direction	Initial Conc. (µM)	Recovery (%)	P_{app} (x 10^{-6} cm/s)			Efflux Ratio
				R1	R2	Average	
MDCK II-WT	Cell-Free	7.9	112	45.7	NA	**45.7**	NA
	Forward	7.9	103	1.4	1.5	**1.5**	4.8
	Reverse	7.6	99	6.7	7.4	**7.1**	
MDCK II-BCRP	Forward	7.8	79	2.1	2.2	**2.1**	6.2
	Reverse	7.8	104	13.5	13.3	**13.4**	
MDCK II-BCRP (10 µM Ko134)	Forward	8.9	103	3.8	5.8	**4.8**	1.4
	Reverse	10.3	96	5.9	8.0	**6.9**	

TAF = tenofovir alafenamide; NA = not applicable

14.3.2. AD-120-2019: In Vitro Assessment of TAF Inhibition of Human OATP1B1, OATP1B3, P-gp, and BCRP

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of GS-7340 Inhibition of Human OATP1B1, OATP1B3, P-gp, and BCRP	Drug-drug interaction (in vitro)	TAF	AD-120-2019

Methods The inhibition potential of TAF of human OATP1B1 and OATP1B3 was assessed in Chinese Hamster Ovary (CHO) cells, either wild type or transfected with the genes encoding human OATP1B1 or OATP1B3. TAF and positive control compound were diluted in assay buffer containing 2 µM Fluo 3. Following removal of media containing Fluo 3, the cells were immediately analyzed for Fluo 3 fluorescence at an excitation of 485 nm and emission of 530 nm.

The inhibition potential of TAF of human P-gp and BCRP was assessed in Madin Darby Canine Kidney (MDCKII) cells, either wild type or transfected with the genes encoding human P-gp or BCRP. The incubation was carried out in cell culture medium (without FBS) containing 10 µM Calcein AM (P-gp) or 1 µM pheophorbide a (PhA) (BCRP). Following removal of media containing calcein AM or PhA, the cells were analyzed immediately for calcein fluorescence at an excitation of 494 nm and an emission of 517 nm or PhA fluorescence at an excitation of 415 nm and an emission of 675 nm.

	Uptake Transporters IC_{50} (µM)		Efflux Transporters IC_{50} (µM)	
Test Compound	**OATP1B1**	**OATP1B3**	**P-gp**	**BCRP**
TAF	>100	>100	>100	>100
Rifampicin	2.4 ± 1.1	1.7 ± 0.4	NA	NA
Verapamil	NA	NA	3.7 ± 3.1	NA
Fumitremorgin C	NA	NA	NA	0.32 ± 0.03

BCRP = breast cancer resistance protein; NA = not applicable; P-gp = P-glycoprotein; TAF = tenofovir alafenamide

14.3.3. AD-120-2013: Effect of GS-9350 on the Bidirectional Permeability of TAF Through Caco-2 Cells

Report Title	Study Type	Test Article	Report Number
Effect of GS-9350 on the Bidirectional Permeability of GS-7340 through Caco-2 Cells	Absorption (in vitro)	TAF	AD-120-2013

Bidirectional Permeability of TAF Through Caco-2 Cells

Inhibitor	Direction	Target Concentration (µM)	Initial Conc. (µM)	Recovery (%)	P_{app} (10^{-6} cm/s)					Efflux Ratio
					Replicate 1	Replicate 2	Replicate 3	Replicate 4	Average	
–	Cell-Free	10	9.61	119	31.6	–	30.1	–	30.8	20
	Forward		9.92	64	0.59	0.69	0.82	0.88	0.74	
	Reverse		8.71	102	18.0	15.1	12.6	14.8	15.1	
GS-9350	Forward	10	11.0	101	3.98	3.56	2.47	2.55	3.14	1.6
	Reverse		11.4	115	6.71	6.18	3.33	3.33	4.89	

Caco-2 = human colonic adenocarcinoma cell line; TAF = tenofovir alafenamide; P_{app} = apparent permeability

14.3.4. AD-120-2022: In Vitro Assessment of TAF as a Substrate for Human OATP1B1 and OATP1B3

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of GS-7340 as a Substrate for Human OATP1B1 and OATP1B3	Drug-drug interaction (in vitro)	TAF	AD-120-2022

Methods: The potential of TAF as a substrate in human OATP1B1 and OATP1B3 was assessed in Chinese Hamster Ovary (CHO) cells, either wild type or transfected with the genes encoding human OATP1B1 or OATP1B3 in the presence and absence of 40 µM rifampicin (OATP inhibitor). Following removal of media, the cell extracts were analyzed by LC/MS/MS.

	Uptake Rate (pmole/min/million cells)		
Test Compound	**TAF**	**TAF + Rifampicin**	**Uptake Ratio**
CHO-WT	9.0	6.0	1.5
CHO-OATP1B1	12.0	6.2	1.9
CHO-OATP1B3	24.1	5.8	4.2
OATP1B1/WT Ratio	1.3	NA	NA
OATP1B3/WT Ratio	2.7	NA	NA

NA = not applicable; OATP = organic anion transporting polypeptide (SLCO or SLC22A gene products); TAF = tenofovir alafenamide;

14.3.5. AD-120-2027: Effects of HIV Protease Inhibitors and Pharmacokinetic Enhancers on TAF Metabolism In Vitro

Report Title	Study Type	Test Article	Report Number
Effects of HIV Protease Inhibitors and Pharmacokinetic Enhancers on the In Vitro Metabolism of GS-7340 in Human Intestinal Subcellular Fraction	Drug-drug interaction (in vitro)	TAF	AD-120-2027

Methods: Duplicate aliquots of 2 µM TAF were incubated with HIV protease inhibitors, atazanavir and darunavir, and the pharmacokinetic enhancers, ritonavir and cobicistat in pooled human intestinal S9 fractions at 37ºC up to 120 minutes. Rates of metabolism (in vitro half-life values) were determined. Analysis was done by LC/MS/MS.

Inhibitor or Enhancer	Concentration (µM)	TAF Intestinal S9 Stability, $t_{½}$ (min)
Vehicle Control	0	24.5 ± 4.1
Atazanavir	25	28.9 ± 5.2
	100	38.9 ± 5.3
Darunavir	25	32.2 ± 5.1
	100	30.8 ± 5.0
Ritonavir	25	19.0 ± 2.8
	100	18.9 ± 1.5
Cobicistat	25	30.1 ± 5.1
	100	32.9 ± 4.8
Dichlorvos (control)	500	>789[a]

TAF = tenofovir alafenamide
a Less than 10% loss of substrate in 120 min

14.3.6. AD-120-2036: In Vitro Assessment of TAF as an Inhibitor of OAT1, OAT3, OCT1, OCT2, MATE1, and BSEP or as a Substrate for OCT1

Report Title	Study Type	Test Article	Report Number
Studies to Determine if Tenofovir Alafenamide (GS-7340) is an Inhibitor of OAT1, OAT3, OCT1, OCT2, MATE1, and BSEP or a Substrate for OCT1	Drug-drug interaction (in vitro)	TAF	AD-120-2036

Methods: The potential for TAF to inhibit the human organic anion and cation uptake transporters, and multidrug and toxin extrusion transporter MATE1 was assessed in vitro using transfected Chinese Hamster Ovary (CHO) cells (for OAT1, OCT1, OCT2, and MATE1), transfected Flp-In 293 cells (for OAT3). The amount of substrate inside the cells was determined by liquid scintillation/fluorescence reader.

Inhibitor or Enhancer	IC_{50} (µM)	Maximum inhibition at 100 µM (% of control)
OCT1	>100	26
OCT2	>100	NA
OAT1	>100	8
OAT3	>100	16
MATE1	>100	34

BSEP = bile salt export protein; MATE1 = multidrug and toxin extrusion protein 1; NA = not applicable; OAT = organic anion transporter; OCT = organic cation transporter; TAF = tenofovir alafenamide;

Report Title	Study Type	Test Article	Report Number
Studies to Determine if Tenofovir Alafenamide (GS-7340) is an Inhibitor of OAT1, OAT3, OCT1, OCT2, MATE1, and BSEP or a Substrate for OCT1	Drug-drug interaction (in vitro)	TAF	AD-120-2036

Methods The potential for TAF to inhibit the bile salt export pump BSEP was assessed in vitro using transfected Chinese hamster ovary (CHO) cells. TAF was incubated with membrane vesicle preparations (total protein: 50 µg/well) and probe substrate, taurocholate (2 µM) in the absence or presence of ATP. The amount of substrate inside the filtered vesicles was determined by liquid scintillation reader.

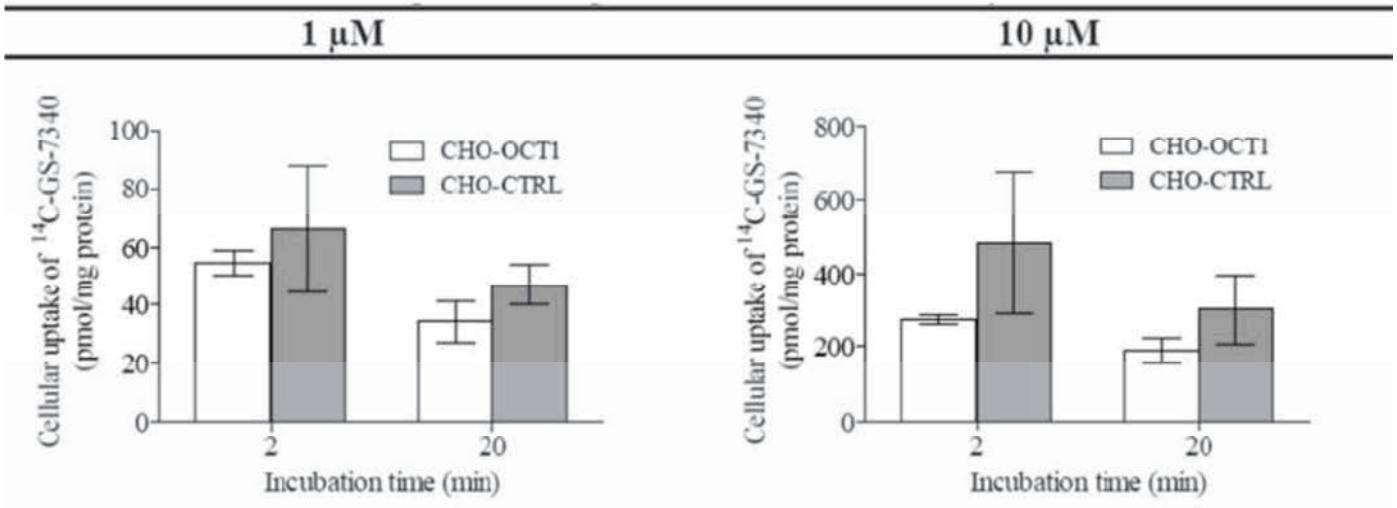
Inhibitor or Enhancer	IC_{50} (µM)	Maximum inhibition at 100 µM (% of control)
BSEP	>100	43

BSEP = bile salt export protein; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Studies to Determine if Tenofovir Alafenamide (GS-7340) is an Inhibitor of OAT1, OAT3, OCT1, OCT2, MATE1, and BSEP or a Substrate for OCT1	Drug-drug interaction (in vitro)	[^{14}C]TAF	AD-120-2036

Methods The interactions of TAF with human cation transporters OCT1 was assessed in vitro using OCT1 transporter-expressed Chinese hamster ovary (CHO) cells. Transporter specific accumulation into OCT1 transporter-expressing cells was investigated at 2 concentrations (1 and 10 µM) and time points (2 and 20 minutes) to determine if TAF is a substrate for this transporter.

Results: TAF was found not to be a substrate for OCT1 based on no transporter specific accumulation of TAF in the OCT1 transporter-expressing cells.



14.3.7. AD-120-2031: Effect of Inhibitors of CatA, CES1, and CYP3A4 on TAF Metabolism In Vitro

Report Title	Study Type	Test Article	Report Number
Effect of Inhibitors of Cathepsin A, Carboxylesterase 1, and CYP3A4 on Metabolism of Tenofovir Alafenamide Fumarate (GS-7340) in Primary Human Hepatocytes	Drug-drug interaction (in vitro)	TAF	AD-120-2031

Methods The effect of inhibitors of cathepsin A (CatA), carboxylesterase 1 (CES1), and CYP3A on TAF activation to the pharmacologically active nucleotide analog diphosphate, tenofovir diphosphate (TFV-DP), was assessed in primary human hepatocytes. The duplicate wells were incubated with 0.5 µM TAF in the presence and absence of the inhibitors (concentrations at 0, 0.08, 0.4, 2, 10, and 50 µM). The amount of TFV-DP formation was analyzed by LC/MS/MS.

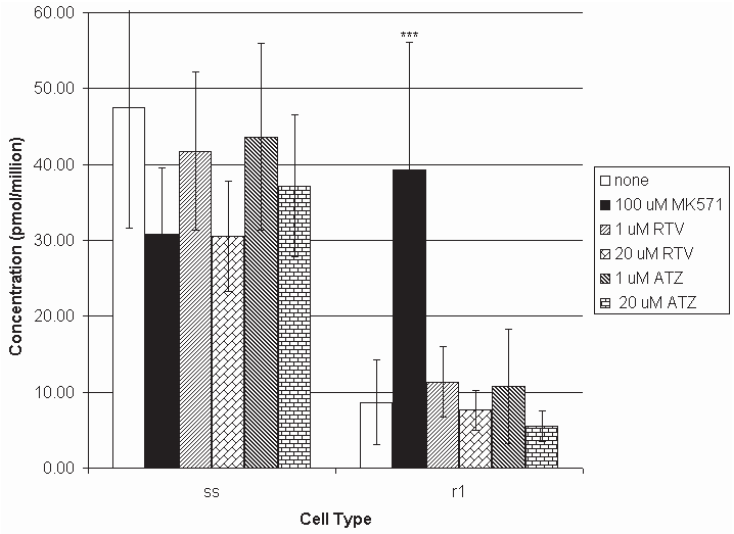
Inhibitor Concentration (µM)	Intracellular TFV-DP (% Control)				
	Telaprevir	Boceprevir	BNPP	COBI	Telaprevir + BNPP
0	100	100	100	100	100
0.08	109	91.2	124	123	132
0.4	93.8	91.1	94.1	98.3	103
2	103	91.7	63.4	100	51.9
10	106	87.2	70.2	88.2	16.1
50	984	100	33.6	87.9	5.30

CatA = cathepsin A; CES1 = carboxylesterase 1; CYP = cytochrome P450 enzyme; TAF = tenofovir alafenamide
Note: Telaprevir and boceprevir are CatA inhibitors; cobicistat is a CYP3A inhibitor; bis(p-nitrophenyl) phosphate (BNPP) is a CES1 inhibitor

14.3.8. AD-104-2001: In Vitro Study of the Potential of TFV to be a Substrate of MRP2 and MRP4 and the Effect of the HIV Protease Inhibitors Atazanavir and Ritonavir

Report Title	Study Type	Test Article	Report Number
Effect of HIV Protease Inhibitors on the Transport of Tenofovir by the Multidrug Resistance Related Proteins 2 and 4	Drug-Drug Interaction	TFV	AD-104-2001

The effects of RTV and ATZ on the accumulation of TFV (1 µM) in MRP4 over-expression cells (r1) and parental cells (SS) were determined. It was found that > 5-fold less TFV accumulated in CEM-R1cells (MRP4 over-expressing) relative to CEM-SS parental cells following. MK571 (100 µM, a potent inhibitor of MRPs) was able to increase TFV levels in CEM-R1 cells to concentrations similar to those in CEM-SS cells. RTV and ATZ (1 and 20 µM) had no significant effect on TFV levels in CEM-SS or –R1 cells.



Statistically significant change in intracellular TFV relative to no cotreatment in CEM-R1 cells (MRP4 overexpressing). Unpaired 2-tailed student's t-test assuming equal variance (***P < 0.001).

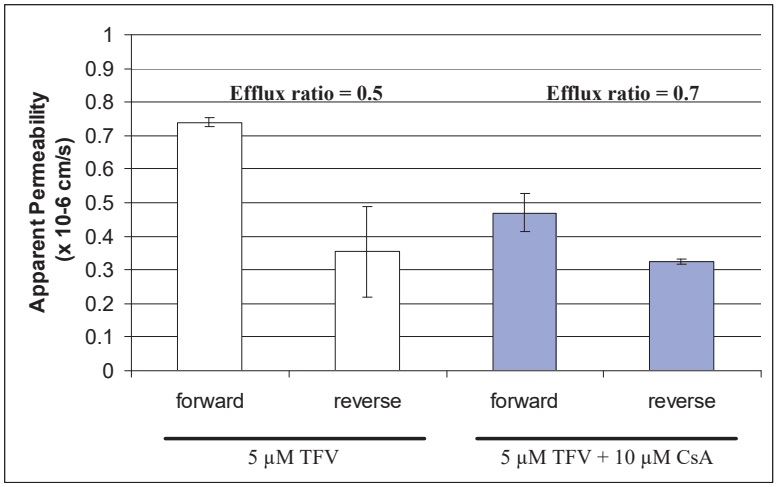
Conclusion: These findings implicate MRP4, but not MRP2, as a transporter potentially contributing to the active tubular secretion of TFV. The results of these studies also suggest that the HIV-PIs ATZ and RTV, even when tested at supra-pharmacological concentrations, are either weak or not inhibitors of transport mediated by MRP2 and MRP4.

14.3.9. AD-104-2002: In Vitro Study of the Potential of TFV to be a Substrate of P-gp and the Effect of other Drugs

Report Title	Study Type	Test Article	Report Number
Lack of a Contribution from P-glycoprotein (P-gp) in the Active Tubular Secretion of Tenofovir	Drug-Drug Interaction	TFV or TDF or tenofovir amidate	AD-104-2002

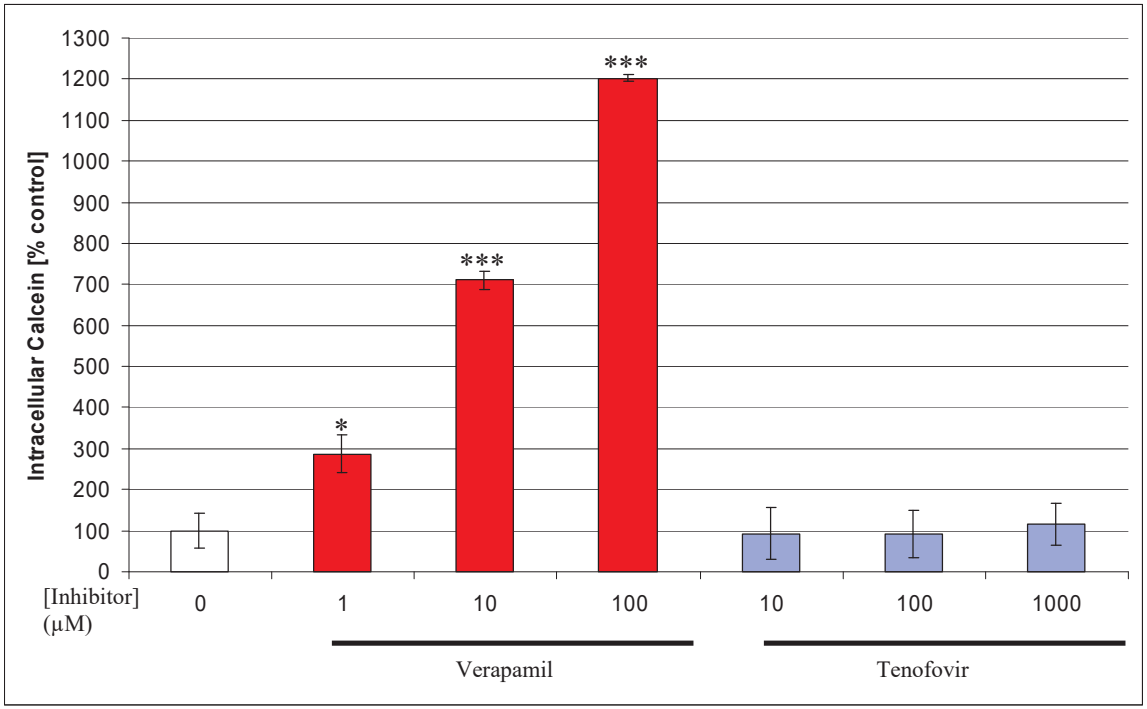
Methods In vitro bidirectional permeability experiments with TFV (5 and 50 µM) in Caco-2 cell monolayers. In vitro inhibition studies of the accumulation of a model multidrug resistance protein (MDR1; P-glycoprotein) substrate (calcein) by TFV (up to 1000 µM) were conducted following incubation of a Madin-Darby canine kidney cell line (MDCK II), both parental and stably transfected with human P-gp, with calcein AM.

Results: The figure below shows the forward and reverse permeability of TFV through Caco-2 cell monolayers, and the calculated efflux ratio in the presence or absence of CsA. TFV had similar forward and reverse permeability that was unaffected by incubation with the P-gp inhibitor CsA (cyclosporine).



Report Title	Study Type	Test Article	Report Number
Lack of a Contribution from P-glycoprotein (P-gp) in the Active Tubular Secretion of Tenofovir	Drug-Drug Interaction	TFV or TDF or tenofovir amidate	AD-104-2002

The figure below displays the effect of TFV and the control P-gp inhibitor, verapamil, on the accumulation of the fluorescent P-gp substrate calcein in MDCK cells transfected with human P-gp. Verapamil significantly inhibited P-gp and resulted in higher intracellular calcein levels with increasing concentration. Tenofovir at concentrations up to 1000 µM did not inhibit the P-gp efflux of calcein.



Conclusion: The observation that TFV has an efflux ratio close to 1, which is not affected by a P-gp inhibitor, in Caco-2 cell monolayers suggests that it is not a P-gp substrate. TFV did not inhibit the transport of a P-gp substrate when tested at suprapharmacological concentrations in MDCK II cells stably transfected with P-gp indicating that it is not a substrate or inhibitor of P-gp.

14.3.10. PC-104-2010: Potential for TFV to be an OAT3 Substrate and the Effect of the Other Drugs

Report Title	Study Type	Test Article	Report Number
Effect of HIV Protease Inhibitors and Other Therapeutics on the Transport of Tenofovir by Human Renal Organic Anion Transporter Type 1 (OAT1)	Drug-Drug Interaction	TFV	PC-104-2010

Methods: The effect of protease inhibitors and other therapeutics on the OAT1 mediated transport of [^{3}H]TFV (1.2 µM) was studied in a stable cell line (CHO-OAT1 cells). The test drugs were examined at concentrations corresponding to 3x, 1x and 0.33x their reported clinical C_{max} values.

Tested drug	Concentration (Fold Clinical C_{max})	Transport of TFV by OAT1 [% control]	
		Serum-free Medium	50% Human Serum[a]
None	-	100	100
Lopinavir	3x	62.5 ± 0.7	113.0 ± 19.8
	1x	87.5 ± 0.7	94.8 ± 1.8
	0.33x	96.0 ± 4.2	109.0 ± 7.1
Lopinavir/ritonavir	3x	55.0 ± 2.8	95.0 ± 5.7
	1x	86.5 ± 0.7	97.0 ± 5.7
	0.33x	109.5 ± 3.5	103.0 ± 0.0
Ritonavir	3x	73.0 ± 7.1	98.5 ± 6.4
	1x	89.5 ± 4.9	97.5 ± 7.8
	0.33x	95.5 ± 4.9	102.5 ± 13.4

Report Title	Study Type	Test Article	Report Number
Effect of HIV Protease Inhibitors and Other Therapeutics on the Transport of Tenofovir by Human Renal Organic Anion Transporter Type 1 (OAT1)	Drug-Drug Interaction	TFV	PC-104-2010

Tested drug	Concentration (fold Clinical C_{max})	Transport of TFV by OAT1 [% control]	
		Serum-free Medium	50% Human Serum[a]
Atazanavir	3x	104.0 ± 0.0	nd[b]
	1x	102.0 ± 2.8	nd
	0.33x	103.5 ± 3.5	nd
Saquinavir	3x	88.5 ± 9.2	102.5 ± 10.6
	1x	90.0 ± 8.5	96.0 ± 7.1
	0.33x	97.5 ± 2.1	98.0 ± 9.9
Nelfinavir	3x	63.5 ± 13.4	90.5 ± 6.4
	1x	78.5 ± 10.6	94.0 ± 1.4
	0.33x	95.0 ± 12.7	107.5 ± 0.7
Amprenavir	3x	78.5 ± 7.8	96.5 ± 12.0
	1x	85.0 ± 8.5	104 ± 9.9
	0.33x	98.0 ± 7.1	109 ± 8.5

Report Title	Study Type	Test Article	Report Number
Effect of HIV Protease Inhibitors and Other Therapeutics on the Transport of Tenofovir by Human Renal Organic Anion Transporter Type 1 (OAT1)	Drug-Drug Interaction	TFV	PC-104-2010

Tested Drug	Concentration (fold Clinical C_{max})	Transport of TFV by OAT1 [% control]	
		Serum-free Medium	50% Human Serum[a]
None	-	100	100
Acyclovir	3x	91 ± 2.8	nd [b]
	1x	94 ± 2.8	nd
	0.33x	101 ± 4.2	nd
Ganciclovir	3x	101 ± 0.0	nd
	1x	92.5 ± 7.8	nd
	0.33x	102.5 ± 0.7	nd
Oseltamivir carboxylate	3x	101 ± 2.8	nd
	1x	102 ± 1.4	nd
	0.33x	118 ± 2.8	nd
Trimethoprim	3x	103.5 ± 6.4	nd
	1x	98.5 ± 4.9	nd
	0.33x	101.5 ± 0.7	nd
Sulfamethoxazole	3x	79.5 ± 6.4	91.5 ± 0.7
	1x	93.5 ± 0.7	98.5 ± 0.7
	0.33x	102 ± 1.4	112 ± 4.2
Amoxicillin	3x	91 ± 5.7	nd
	1x	91.5 ± 3.5	nd
	0.33x	94 ± 8.5	nd

Report Title	Study Type	Test Article	Report Number
Effect of HIV Protease Inhibitors and Other Therapeutics on the Transport of Tenofovir by Human Renal Organic Anion Transporter Type 1 (OAT1)	Drug-Drug Interaction	TFV	PC-104-2010

Tested Drug	Concentration (fold Clinical C_{max})	Transport of TFV by OAT1 [% control]	
		Serum-free Medium	50% Human Serum[a]
Ibuprofen	3x	7[c]	99.5 ± 2.1
	1x	19[c]	102.5 ± 0.7
	0.33x	32[c]	114.5 ±4.9
Acetaminophen	3x	78.5 ± 3.5	94.5 ± 3.5
	1x	88 ± 1.4	94.5 ± 9.2
	0.33x	97 ± 7.1	106.5 ± 3.5

14.3.11. PC-104-2011: Potential for TFV to be an OAT3 Substrate and the Effect of HIV-PIs

Report Title	Study Type	Test Article	Report Number
Effect of HIV Protease Inhibitors on the Transport of TFV by Human Renal Organic Anion Transporter Type 3 (OAT3)	Drug-Drug Interaction	TFV	PC-104-2011

Type of Study: In vitro study was conducted in appropriate cell lines expressing hAOT3 to define the role of OAT3 in tenofovir (TFV) transport and to assess the effect of HIV protease inhibitors (HIV-PIs) on the OAT3 transport of TFV.

Methods: The effect of HIV-PIs on the OAT3 mediated transport of [^{3}H]TFV (1.2 µM) was studied in a stable cell line (BHK-OAT3 cells). HIV-PIs were tested at concentrations corresponding to 2x, 1x, and 0.5x their reported clinical C_{max} values. The kinetics of [^{3}H]TFV and [^{3}H]estrone sulphate transport were also determined in BHK-OAT3 cells.

Substrate	K_m [µM]	V_{max} [pmol/10^6 cells/min]	Transport efficiency (V_{max}/K_m)
Tenofovir (n=3)	767 ± 145	31.6 ± 8.0	0.043 ± 0.016
Estrone sulfate (n=4)	3.0 ± 1.2	2.9 ± 1.2	0.97 ± 0.15

Report Title	**Study Type**	**Test Article**	**Report Number**
Effect of HIV Protease Inhibitors on the Transport of TFV by Human Renal Organic Anion Transporter Type 3 (OAT3)	Drug-Drug Interaction	TFV	PC-104-2011

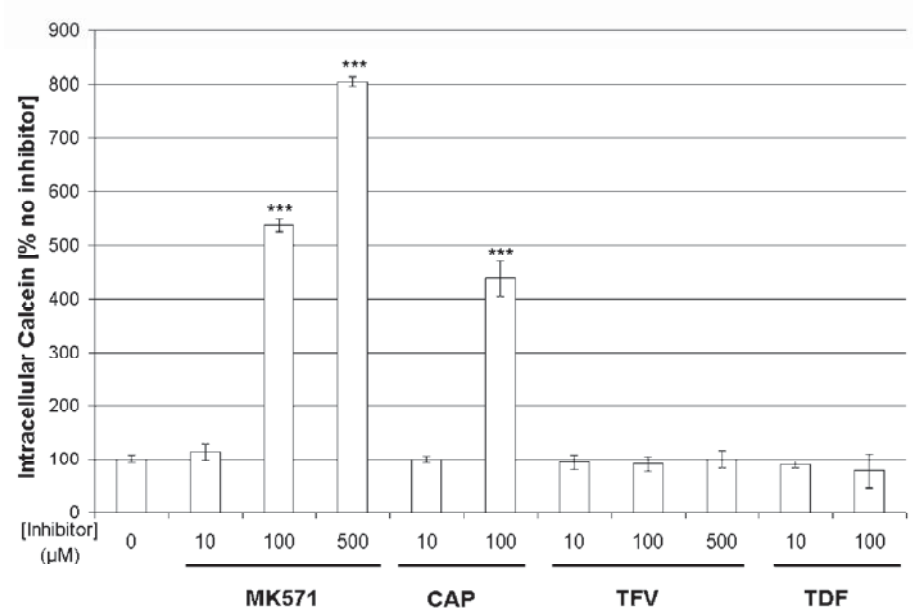
Tested PI	**Concentration [fold Clinical C_{max}]**	**Transport of TFV by OAT3 [% control]**	
		Serum-free Medium	**40% Human serum**
No inhibitor	-	100	100
Ritonavir	0.5x	57.7 ± 5.1	87.1 ± 6.7
	1x	38.4 ± 5.7	65.9 ± 8.8
	2x	21.1 ± 3.2	59.4 ± 7.0
Lopinavir/ritonavir	0.5x	78.9 ± 2.6	93.3 ± 12.2
	1x	63.5 ± 8.7	92.1 ± 6.8
	2x	46.1 ± 5.1	78.5 ± 9.1
Atazanavir	0.5x	102.3 ± 1.6	103.8 ± 14.9
	1x	94.7 ± 7.8	98.5 ± 4.4
	2x	90.8 ± 2.4	97.1 ± 7.7
Nelfinavir	0.5x	84.6 ± 2.3	102.0 ± 10.2
	1x	73.1 ± 3.2	101.6 ± 11.2
	2x	47.7 ± 2.3	99.4 ± 9.8
Saquinavir	0.5x	98.3 ± 0.1	99.2 ± 8.7
	1x	95.1 ± 3.6	98.3 ± 3.7
	2x	98.8 ± 3.7	101.1 ± 13.2
Amprenavir	0.5x	87.8 ± 5.6	97.0 ± 2.7
	1x	81.4 ± 3.1	90.3 ± 5.2
	2x	78.7 ± 2.4	86.0 ± 1.5

Conclusion: TFV was found to be a low affinity substrate for OAT3 (K_M 767 uM). When adjusted for plasma protein binding and tested at their clinical C_{max} concentration, HIV-PIs had no significant effect on TFV transport by OAT3.

14.3.12. PC-104-2014: Effect of TFV on the Activity of Human Multidrug Resistance Related Protein MRP1

Report Title	Study Type	Test Article	Report Number
Lack of a Contribution from MRPl in Tubular Re-absorption of TFV	Drug-Drug Interaction	TFV	PC-104-2014

Methods: Inhibition of the accumulation of a model MRP1 substrate (calcein) in a Madin-Darby canine kidney cell line (MDCK II) stably transfected with human MRP1, and treated with calcein-AM. Control inhibitors, MK571 and CAP (caffeic acid phenethyl ester), were tested in parallel.



Conclusion: Suprapharmacological concentrations of TFV did not inhibit the transport of an MRP1 substrate in MDCK II cells stably transfected with MRP1.

14.3.13. AD-104-2012: Effects of TFV on Transport by Human OCT2 and MATE1

Report Title	Study Type	Test Article	Report Number
In Vitro Inhibition Studies of Tenofovir with Human OCT2 and MATE1 Transporters	Drug-Drug Interaction	TFV	AD-104-2012

Type of Study: In vitro study of the effect of TFV on the activity of the human renal transporters, OCT1 and MATE1, expressed individually in CHO cells.

Methods: The model substrate was [^{14}C]triethylamine (TEA, 3.6 µM) and cell monolayers were exposed for 5 minutes (OCT2) or 20 minutes (MATE1) before being washed and cell-associated radioactivity determined. Results were compared to those obtained in nontransfected CHO cells. Positive control inhibitors (100 µM verapamil for OCT2 and 100 µM quinidine for MATE1) were tested in parallel.

Results: In the absence of inhibitors, accumulation of TEA in transfected cells was 25-fold higher (OCT2) and 18-fold higher (MATE1) than parental CHO cells. Positive control inhibitors reduced the transporter-specific accumulation of TEA to 8.68 ± 2.56% (OCT2) and 3.70 ± 0.62% (MATE1) of the vehicle control, confirming sensitivity to inhibitors. At concentrations of TFV up to 300 µM there was little or no effect on TEA accumulation.

Inhibitor	Transporter	IC_{50} (µM)	Maximum inhibition
TFV	OCT2	> 300	< 10%
	MATE1	> 300	~20%

CHO = Chinese hamster ovary; MATE1 = multidrug and toxin extrusion protein 1; OCT2 = organic cation transporter 2

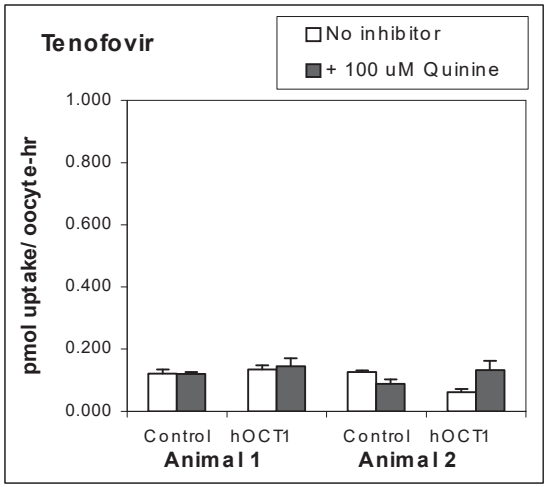
14.3.14. PC-103-2001: Interactions of TFV with Human OAT3, OCT1, and OCT2

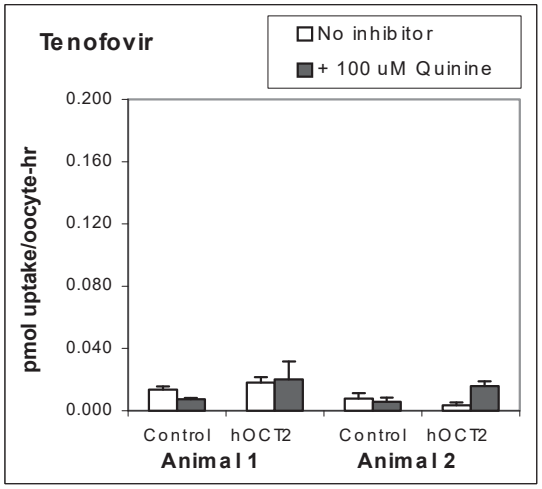
Report Title	Study Type	Test Article	Report Number
In Vitro Interactions of Acyclic Nucleoside Phosphonate Analogs with Human Organic Cation and Anion Transporters	Drug-Drug Interaction	TFV	PC-103-2001

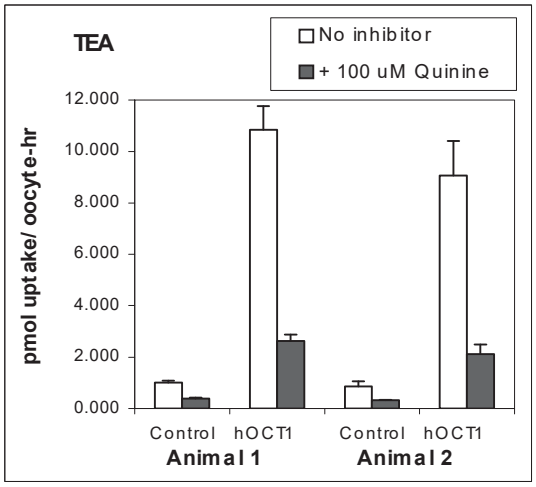
Type of Study: In vitro study to evaluate the interactions of tenofovir (TFV) with human organic anion transporter OAT3 and human cation transporters OCT1 and OCT2.

Methods: The interactions of TFV with OCT1, OCT2, and OAT3 were studied in a Xenopus oocyte expression system through uptake transport of [^{3}H]TFV (10 μM) and appropriate control substrates ([^{14}C]TEA triethylamine, 100 μM for OCT1 and OCT2, and [^{3}H]estrone sulphate (100 nM) for OAT3). Inhibition studies were also conducted for OAT3 by measuring the uptake of [^{3}H]estrone sulphate (100 μM) in the presence TFV and the positive control inhibitor, probenicid, at concentrations from 50 to 1000 μM.

Results (OCT1 and OCT2): The figures below show the results of uptake studies of [^{3}H]TFV (10 μM) for OCT1 and OCT2 in comparison to a water-injected oocytes (Control) in the presence and absence of the inhibitor quinine. The positive control substrate, TEA, showed the expected high level of transport. TFV was shown not to be a substrate for OCT1 and OCT2.

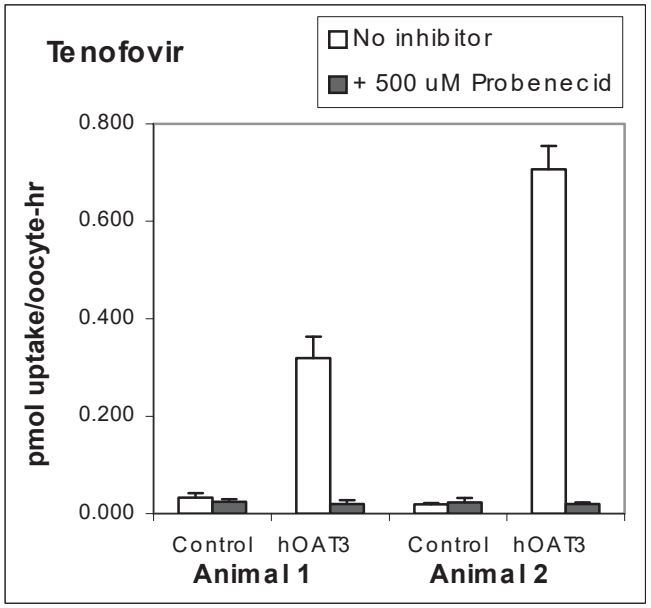


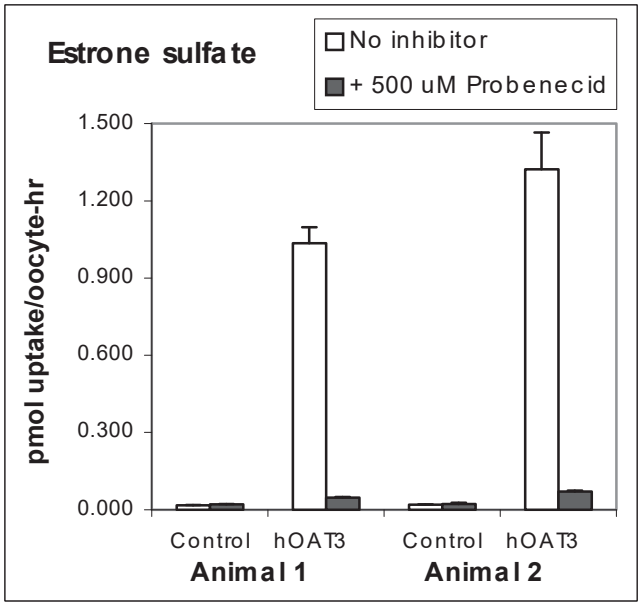




Report Title	Study Type	Test Article	Report Number
In Vitro Interactions of Acyclic Nucleoside Phosphonate Analogs with Human Organic Cation and Anion Transporters	Drug-Drug Interaction	TFV	PC-103-2001

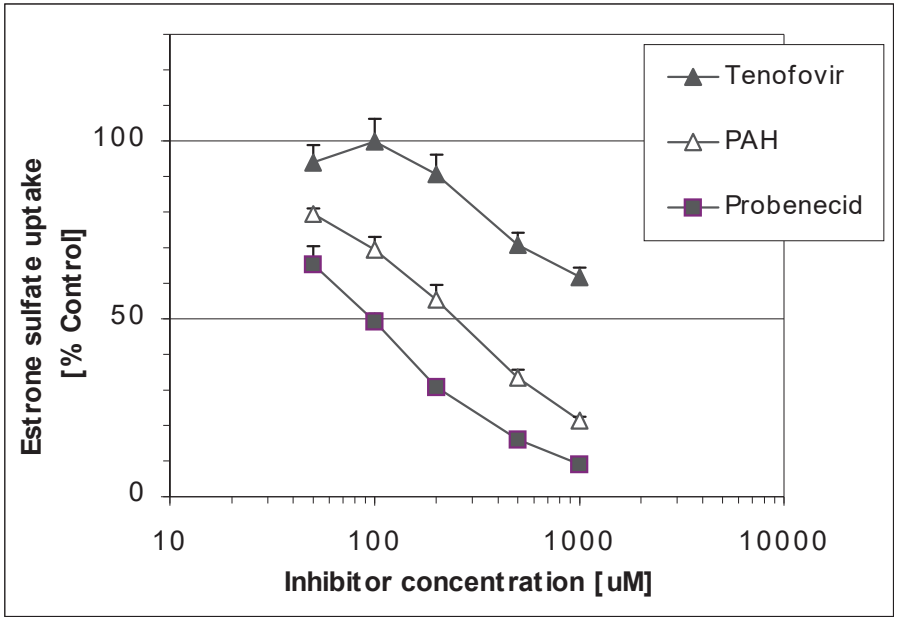
Results (OAT3): The figures below display the results of uptake studies of [^{3}H]TFV (10 µM) for human OAT3 in comparison to water-injected oocytes (Control) in the presence and absence of the control inhibitor, probenecid. The positive control substrate [^{3}H]estrone sulphate showed the expected high level of transport. TFV was shown to be a substrate for OAT3 transport.





Report Title	Study Type	Test Article	Report Number
In Vitro Interactions of Acyclic Nucleoside Phosphonate Analogs with Human Organic Cation and Anion Transporters	Drug-Drug Interaction	TFV	PC-103-2001

The affinity of TFV for OAT3 was assessed indirectly through inhibition of the transport of [^{3}H]estrone sulphate in comparison to the effect of probenicid and para-aminohippurate (PAH). The results are shown in the figure below. No inhibition of OAT3 was observed in the presence of up to 100 µM TFV indicating a low affinity interaction between TFV and OAT3.



Conclusion: This study demonstrated that TFV was not a substrate for human cation transporters OCT1 and OCT2. TFV was shown to be a low affinity substrate for OAT3.

14.3.15. AD-120-2035: Effect of Cyclosporin A Pretreatment on Pharmacokinetics of TAF in Dogs

Report Title	Study Type	Test Article	Report Number
Effect of Cyclosporin A pretreatment on Pharmacokinetics of Tenofovir Alafenamide in dogs	Drug-drug interaction (in vivo)	TAF	AD-120-2035

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/3
Method of Administration:	Intravenous infusion
Dose (mg/kg):	0.5
Sample	Plasma/PBMC
Assay:	LC/MS/MS

	Plasma		PBMC		
			- Phosphatase	+ Phosphatase	–
Parameter	**TAF**	**TFV**	**TFV**	**TFV**	**TFV-DP**
T_{max} (h)	0.41	0.90	0.48	2.32	6.00
C_{max} (µg/mL)	0.47	0.03	2.96	5.25	7.24
$t_{½}$ (h)	0.12	>24	NC	>24	>24
AUC_{0-t} (µg•h/mL)	0.23	0.15	12.3	78.7	137
CL (L/h/kg)	2.23	NA	NA	NA	NA

F = female; M = male; NA = not applicable; NC = not calculable; PBMC = peripheral blood mononuclear cell; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-DP = tenofovir diphosphate

Report Title	Study Type	Test Article	Report Number
Effect of Cyclosporin A pretreatment on pharmacokinetics of Tenofovir Alafenamide in dogs	Drug-drug interaction (in vivo)	TAF	AD-120-2035

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/3
Method of Administration:	Oral gavage
Dose (mg/kg):	2
Feeding Condition:	Fasted
Vehicle / Formulation:	0.9% sodium chloride in 50 mM ammonium acetate, pH 5.5
Assay:	LC/MS/MS

	Plasma		PBMC		
			- Phosphatase	+ Phosphatase	–
	TAF	TFV	TFV	TFV	TFV-DP
T_{max} (h)	0.14	0.67	1.00	1.00	8.70
C_{max} (µg/mL)	0.05	0.10	0.12	0.75	1.81
$t_{½}$ (h)	0.21	>24	NC	>24	>24
AUC_{0-t} (µg•h/mL)	0.01	0.33	NA	10.8	31.2
F%	1.67	NA	NA	NA	NA

F = female; M = male; NA = not applicable; NC = not calculable; PBMC = peripheral blood mononuclear cell; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-DP = tenofovir diphosphate

Report Title	Study Type	Test Article	Report Number
Effect of Cyclosporin A pretreatment on pharmacokinetics of Tenofovir Alafenamide in dogs	Drug-drug interaction (in vivo)	TAF	AD-120-2035

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/3
Method of Administration:	Oral gavage
Dose (mg/kg):	2
Co-administered CsA Dose (mg/kg):	75
Feeding Condition:	Fasted
Vehicle / Formulation:	50 mM citrate, pH 5
Assay:	LC/MS/MS

	Plasma (Pretreated with 75 mg Cyclosporin A)		PBMC (Pretreated with 75 mg Cyclosporin A)		
			- Phosphatase	+ Phosphatase	–
	TAF	TFV	TFV	TFV	TFV-DP
T_{max} (h)	0.25	1.00	1.00	1.00	3.00
C_{max} (µg/mL)	0.39	0.05	2.10	4.91	3.15
$t_{½}$ (h)	0.15	>24	>24	>24	>24
AUC_{0-t} (µg•h/mL)	0.15	0.38	9.39	75.8	59.5
F%	16.6	NA	NA	NA	NA

F = female; M = male; NA = not applicable; PBMC = peripheral blood mononuclear cell; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-DP = tenofovir diphosphate

14.4. EVG/COBI/FTC/TFV

14.4.1. AD-236-2001: Inhibition Potential of EVG, FTC, TFV, and COBI with Human OCT2 and MATE1 Transporters

Report Title	Study Type	Test Article	Report Number
In Vitro Inhibition Studies of Elvitegravir, Emtricitabine, Tenofovir, and Cobicistat (Quad) with Human OCT2 and MATE1 Transporters	Drug-drug interaction (in vitro)	EVG, COBI, FTC, TFV	AD-236-2001

Methods The potential for EVG, COBI, FTC, and TFV to inhibit the human organic cation uptake transporters OCT2 and multidrug and toxin extrusion transporter MATE1 was assessed in vitro using transfected Chinese Hamster Ovary (CHO) cells lines stably expressing human OCT2 or MATE1 protein. The amount of cell-associated radioactivity was determined by liquid scintillation counting. IC_{50} values were calculated by analysis of the concentration-dependent reduction of the fractional transport activity by the test compounds, with 100% transport activity being that seen with the vehicle control. IC_{50} values were determined by nonlinear regression using GraphPad Prism 5.0.

Inhibitor or Enhancer	Transporter	IC_{50} (µM)	Maximum inhibition at 100 µM (% of control)
Elvitegravir	OCT2	>20	31
	MATE1	2.0	98
Emtricitabine	OCT2	>100	18
	MATE1	>100	ND
Tenofovir	OCT2	>300	ND
	MATE1	>300	20
Cobicistat	OCT2	14.4	70
	MATE1	1.87	98

COBI = cobicistat; EVG = elvitegravir; FTC = emtricitabine; ND = not determined; TAF = tenofovir alafenamide; TFV = tenofovir
Note: Maximum concentration tested was 300 µM for tenofovir, 100 µM for emtricitabine and cobicistat, and 20 µM for elvitegravir

14.4.2. AD-236-2003: Assessment of Inhibition of Human P-gp and BCRP by EVG, FTC, and TFV In Vitro

Report Title	Study Type	Test Article	Report Number
In Vitro Inhibition of Human P-gp and BCRP by Elvitegravir, Emtricitabine and Tenofovir	Drug-drug interaction (in vitro)	EVG, COBI, FTC, TFV	AD-236-2003

Methods: The inhibition of the ATP-Binding Cassette (ABC) efflux P-glycoprotein (P-gp) transporter and breast cancer resistance protein (BCRP) by EVG, COBI, FTC, and TFV was assessed in vitro using the individual transporters and fluorescent model substrates. Verapamil was the positive control inhibited P-gp activity and fumitremorgin C was the positive control inhibitor in BCRP inhibition assay.

	Efflux Transporter IC_{50} (µM)	
Test Compound	**P-gp**	**BCRP**
Elvitegravir	69.7 ± 5.4	88.9 ± 16.0
Emtricitabine	>100	>100
Tenofovir	> 1000	>100
Cobicistat	36 ± 10	59 ± 28
Verapamil	5.2 ± 1.2	NA
Fumitremorgin C (FTC)	NA	0.37 ± 0.18

COBI = cobicistat; EVG = elvitegravir; FTC = emtricitabine; TFV = tenofovir; NA = not applicable

14.4.3. AD-236-2004: Bidirectional Permeability of EVG, FTC, TFV, and COBI through Monolayer of P-gp (MDR1)-Overexpressing Cells

Report Title	Study Type	Test Article	Report Number
Bidirectional Permeability of Elvitegravir, Emtricitabine, Tenofovir, and Cobicistat (Quad) through Monolayers of P-glycoprotein Overexpressing Cells	Drug-drug interaction (in vitro)	FTC	AD-236-2004

Bidirectional Permeability of FTC Through and MDR1 Transfected MDCKII Cells

Cell Type	Direction	Initial Conc. (µM)	Recovery (%)	P_{app} (10^{-6} cm/s)			Efflux Ratio
				Replicate 1	Replicate 2	Average	
MDCKII-WT	Cell-Free	11.2	49	15.3	–	15.3	–
	Forward	9.9	60	0.38	0.34	0.36	2.3
	Reverse	6.9	167	0.75	0.86	0.81	
MDCKII-MDR1	Forward	11.5	79	0.22	0.16	0.19	3.2
	Reverse	10.5	95	0.77	0.42	0.60	
MDCKII-MDR1 (10 µM CsA)	Forward	12.7	88	0.12	0.29	0.21	3.3
	Reverse	9.8	121	0.60	0.76	0.68	

CsA = cyclosporin A; FTC = emtricitabine; MDCKII = Madin-Darby canine kidney cells; MDR1 = P-glycoprotein (P-gp, ABCB1 gene product); P_{app} = apparent permeability; WT = wild type

Report Title	Study Type	Test Article	Report Number
Bidirectional Permeability of Elvitegravir, Emtricitabine, Tenofovir, and Cobicistat (Quad) through Monolayers of P-glycoprotein Over-expressing Cells	Drug-drug interaction (in vitro)	TFV	AD-236-2004

Bidirectional Permeability of TFV Through and MDR1 Transfected MDCKII Cells

Cell Type	Direction	Initial Conc. (μM)	Recovery (%)	P_{app} (10^{-6} cm/s)			Efflux Ratio
				Replicate 1	Replicate 2	Average	
MDCKII-WT	Cell-Free	9.8	88	13.3	–	13.3	–
	Forward	9.5	104	0.18	0.11	0.15	1.5
	Reverse	13.1	95	0.19	0.25	0.22	
MDCKII-MDR1	Forward	16.0	94	0.10	0.12	0.11	1.0
	Reverse	16.8	92	0.07	0.15	0.11	
MDCKII-MDR1 (10 μM CsA)	Forward	13.2	83	0.12	0.07	0.10	1.5
	Reverse	11.0	82	0.14	0.15	0.14	

CsA = cyclosporin A; MDCKII = Madin-Darby canine kidney cells; MDR1 = P-glycoprotein (P-gp, ABCB1 gene product); Papp = apparent permeability; TFV = tenofovir; WT = wild type

14.4.4. AD-236-2005: Bidirectional Permeability of EVG, FTC, TFV, and COBI through Monolayer of BCRP-Overexpressing Cells

Report Title	Study Type	Test Article	Report Number
Bidirectional Permeability of Elvitegravir, Emtricitabine, Tenofovir, and Cobicistat (Quad) through Monolayers of BCRP Over-expressing Cells	Drug-drug interaction (in vitro)	FTC	AD-236-2005

Bidirectional Permeability of FTC Through and BCRP Transfected MDCKII Cells

Cell Type	Direction	Initial Conc. (µM)	Recovery (%)	P_{app} (10^{-6} cm/s)			Efflux Ratio
				Replicate 1	Replicate 2	Average	
MDCKII-WT	Cell-Free	11.2	49	15.3	–	15.3	–
	Forward	9.9	60	0.38	0.34	0.36	2.3
	Reverse	6.9	167	0.75	0.86	0.81	
MDCKII-BCRP	Forward	10.7	113	0.37	0.51	0.39	3.7
	Reverse	10.2	118	1.25	1.61	1.43	
MDCKII- BCRP (10 µM ko134)	Forward	13.1	61	0.25	0.33	0.29	4.8
	Reverse	9.3	106	1.37	1.42	1.39	

BCRP = breast cancer resistance protein; FTC = emtricitabine; MDCKII = Madin-Darby canine kidney cells; P_{app} = apparent permeability; WT = wild type

Report Title	Study Type	Test Article	Report Number
Bidirectional Permeability of Elvitegravir, Emtricitabine, Tenofovir, and Cobicistat (Quad) through Monolayers of BCRP Over-expressing Cells	Drug-drug interaction (in vitro)	TFV	AD-236-2005

Bidirectional Permeability of TFV Through and BCRP Transfected MDCKII Cells

Cell Type	Direction	Initial Conc. (µM)	Recovery (%)	P_{app} (10^{-6} cm/s)			Efflux Ratio
				Replicate 1	Replicate 2	Average	
MDCKII-WT	Cell-Free	9.8	88	13.3	–	13.3	–
	Forward	9.5	104	0.18	0.11	0.15	1.5
	Reverse	13.1	95	0.19	0.25	0.22	
MDCKII-BCRP	Forward	12.1	92	0.17	0.14	0.16	2.3
	Reverse	13.1	81	0.40	0.32	0.36	
MDCKII-BCRP (10 µM CsA)	Forward	11.2	91	0.18	0.21	0.19	3.4
	Reverse	11.2	97	0.85	0.45	0.65	

BCRP = breast cancer resistance protein; CsA = cyclosporin A; MDCKII = Madin-Darby canine kidney cells; P_{app} = apparent permeability; TFV = tenofovir; WT = wild type

14.4.5. AD-236-2006: Assessment of Inhibition of Human OATP1B1 and OATP1B3 by FTC and TFV

Report Title	Study Type	Test Article	Report Number
In Vitro Inhibition of Human OATP1B1 and OATP1B3 by Emtricitabine and Tenofovir	Drug-drug interaction (in vitro)	FTC, TFV	AD-236-2006

Methods The inhibition potential of test compounds of human OATP1B1 and OATP1B3 was assessed in Chinese hamster ovary (CHO) cells, either wild type or transfected with the genes encoding human OATP1B1 or OATP1B3. The test compounds and positive control compound were diluted in assay buffer containing 2 µM Fluo 3. Following removal of media containing Fluo 3, the cells were immediately analyzed for Fluo 3 fluorescence at an excitation of 485 nm and emission of 530 nm. Rifampicin was used as positive control.

	Influx Transporters IC_{50} (µM)	
Test Compound	**OATP1B1**	**OATP1B3**
Elvitegravir	>2	0.44 ± 0.22
Emtricitabine	>100	>100
Tenofovir	> 100	>100
Cobicistat	3.50 ± 0.72	1.88 ± 0.76
Rifampicin	1.3 ± 0.8	3.0 ± 0.7

FTC = emtricitabine; OATP = organic anion transporting polypeptide; TFV = tenofovir

14.4.6. AD-236-2007: Assessment of Inhibition of EVG, FTC, TFV, and COBI with Human OAT1, OAT3 and MRP4 Transporters

Report Title	Study Type	Test Article	Report Number
In Vitro Inhibition Studies of Quad Components with Human OAT1, OAT3 and MRP4 Transporters	Drug-drug interaction (in vitro)	EVG, COBI, FTC, TDF	AD-236-2007

Methods The potential for test compounds to inhibit the human organic anion uptake transporters (OAT1 and OAT3) and the apically expressed human multidrug resistance related protein 4 (MRP4) was assessed in vitro. OAT1 and OAT3 cellular uptake assay was performed on Chinese hamster ovary (CHO) cells and human embryonic kidney (HEK293) cells with FlpIn technology (FlpIn293) stably transfected with OAT1 and OAT3, respectively. In MRP4 vesicular transport assay, the test compounds were incubated with membrane vesicle preparations (total protein: 50 µg/well) in the absence or presence of ATP. The amount of substrate inside the cells was determined by liquid scintillation counting.

		Emtricitabine		Elvitegravir		Cobicistat		Tenofovir	
Transporter	Probe Substrate	IC_{50} (µM)	Maximum inhibition (%)	IC_{50} (µM)	Maximum inhibition (%)	IC_{50} (µM)	Maximum inhibition (%)	IC_{50} (µM)	Maximum inhibition (%)
OAT1	PAH	>100	40	>20	No Inhibition	>100	140 activation	33.8	NA
OAT3	E3S	>100	No Inhibition	>20	14	>100	No Inhibition	770	NA
MRP4	E217βG	>100	No Inhibition	>20	19	20.7	~92	>1,000	NA

COBI = cobicistat; EVG = elvitegravir; FTC = emtricitabine; TFV = tenofovir; PAH = ^{3}H- para-aminohippuric acid; E3S = ^{3}H-estrone-3-sulfate; E217βG = estradiol-17-beta-glucuronide; OAT = organic anion transporter; NA = not applicable

Note: Maximum concentrations used for elvitegravir, emtricitabine, cobicistat and tenofovir were 20, 100, 100, and 1000 µM, respectively.
Cobicistat results were also reported in report AD-216-2105.

14.4.7. AD-236-2008: Assessment of Inhibition of EVG, COBI, FTC, and TFV with Human OCT1 and BSEP Transporters

Report Title	Study Type	Test Article	Report Number
In Vitro Inhibition Studies of Stribild Components with Human OCT1 and BSEP Transporters	Drug-drug interaction (in vitro)	EVG, COBI, FTC, TFV	AD-236-2008

Methods The potential for test compounds to inhibit the human organic cation uptake transporter (OCT1) and bile salt export pump (BSEP) was assessed in vitro using transfected Chinese Hamster Ovary (CHO) cells. In OCT2 cellular uptake assay, plated cells were washed with Krebs-Henseleit buffer and were then exposed for 10 minutes to the same buffer containing [^{14}C]-tetraethylammonium chloride (TEA) substrate (3.6 µM) and test compounds. In BSEP vesicular transport assay, was incubated with membrane vesicle preparations (total protein: 50 µg/well) and probe substrate, taurocholate (2 µM) in the absence or presence of ATP. The amount of substrate inside the cells was determined by liquid scintillation counting for both assays.

	Elvitegravir		Emtricitabine		Tenofovir		Cobicistat		Ritonavir	
Transporter	IC_{50} (µM)	Maximum inhibition (%)	IC_{50} (µM)	Maximum inhibition (%)	IC_{50} (µM)	Maximum inhibition (%)	IC_{50} (µM)	Maximum inhibition (%)	IC_{50} (µM)	Maximum inhibition (%)
OCT1	>20	30	>100	No Inhibition	>100	No Inhibition	14.7	76	~20	49
BSEP	>20	33	>100	No Inhibition	>100	No Inhibition	6.5	97	1.8	95.3

BSEP = bile salt export pump; COBI = cobicistat; EVG = elvitegravir; FTC = emtricitabine; OCT = organic cation transporter; Stribild = fixed-dose combination of elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate; TFV = tenofovir
Note: Maximum concentrations used for elvitegravir, emtricitabine, cobicistat, tenofovir and ritonavir were 20, 100, 100, 100, and 20 µM, respectively.

14.4.8. AD-236-2010: Interaction of FTC with Human OAT1 and OAT3 Transporters

Report Title	Study Type	Test Article	Report Number
In vitro Interaction Study of Emtricitabine with the Human OAT1 and OAT3 Uptake Transporters	Drug-drug interaction (in vitro)	FTC	AD-236-2010

Methods The potential for test compounds that as substrates for the renal uptake transporters organic anion transporter (OAT1 and OAT3) was assessed in vitro using wild-type and transfected cell-lines. The transporter specific uptake of emtricitabine was determined using cells overexpressing the OAT1 and OAT3 uptake transporters as well as the control (parental) cells. The test article was be incubated at 37±1°C at final concentrations of 1 and 10 µM. An additional experiment was performed in OAT3 overexpressing cells in the presence and absence of probenecid, a known inhibitor of the transporter. The amount of substrate inside the cells was determined by LC/MS method.

Transporter	Condition (µM/min)	Fold Accumulation
OAT1	1/2	1.59
	1/20	0.66
	10/2	1.51
	10/20	1.45
OAT3	1/2	2.09
	1/20	1.82
	10/2	2.32
	10/20	2.13
	10/2	2.71 (Emtricitabine Alone)
	10/2	1.02 (Emtricitabine + Probenecid)

FTC = emtricitabine; LC/MS = high performance liquid chromatography coupled to tandem mass spectrometry; OAT = organic anion transporter

14.4.9. AD-236-2011: Interaction of FTC and TFV with Human OCT2 Uptake Transporters

Report Title	Study Type	Test Article	Report Number
In vitro Interaction Study of Emtricitabine and Tenofovir with the Human OCT2 Uptake Transporter	Drug-drug interaction (in vitro)	FTC, TFV	AD-236-2011

Methods The potential for test compounds that as substrates for the renal uptake transporter organic cation transporter (OCT2) was assessed in vitro using wild-type and transfected Chinese Hamster Ovary (CHO) cells. The transporter specific uptake of emtricitabine and TFV was determined using cells overexpressing the OCT2 uptake transporters as well as the control (parental) cells. The test articles were incubated at 37±1°C at final concentrations of 1 and 10 µM. The amount of substrate inside the cells was determined by LC/MS method.

	Fold Accumulation	
Condition (µM/min)	**Emtricitabine**	**Tenofovir**
1/2	2.35[a]	1.24
1/20	1.12	1.37
10/2	1.10	0.82
10/20	1.82	0.98

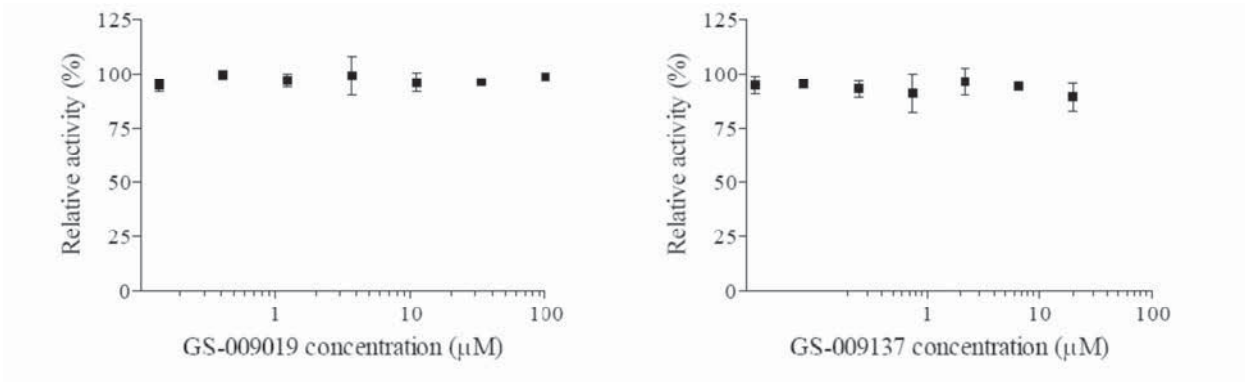
FTC = emtricitabine; LC/MS = high performance liquid chromatography coupled to tandem mass spectrometry; OCT = organic cation transporter; TFV = tenofovir
a Considered outlier

14.4.10. AD-236-2012: Assessment of Interaction of EVG and FTC with Human MRP2 Transporters

Report Title	Study Type	Test Article	Report Number
In Vitro Inhibition of Human MRP2 ABC (Efflux) Transporter by Stribild Components Elvitegravir and Emtricitabine	Drug-drug interaction (in vitro)	EVG, FTC	AD-236-2012

Methods	The inhibition potential of test compounds with human MRP2 ABC (efflux) transporters was assessed in vitro using MRP2 containing vesicles and model substrate estradiol-17-beta-glucuronide (E217bG). The vesicular transport assay was performed with cell membrane vesicles containing the human MRP2 efflux transporter. The MRP2 transporter was expressed in Spodoptera frugiperda (Sf9) ovarian cells. The isolated membrane vesicles were prepared and characterized. The specificity of the interaction was confirmed using control membrane vesicles in the negative control experiment. The concentration of test articles was 0 and the highest applied test article concentration. The amount of substrate inside the filtered vesicles was determined by liquid scintillation.
Results	Elvitegravir and emtricitabine did not influence the MRP2-mediated E217βG transport when tested at concentrations up to 20 µM and 100 µM, respectively.

Effect of FTC and EVG on the MRP2-Mediated E217βG Transport



EVG = elvitegravir (GS-009137), FTC = emtricitabine (GS-009019); MRP2 = multidrug resistance-associated protein-2 (ABCC2, cMOAT); Stribild = fixed-dose combination of elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate

14.4.11. AD-236-2013: Interaction of FTC with Human MRP2 Transporters

Report Title	Study Type	Test Article	Report Number
In Vitro Interaction Study of Emtricitabine with the Human MRP2 ABC (Efflux) Transporter	Drug-drug interaction (in vitro)	FTC	AD-236-2013

Methods The potential for test compound that as substrates for human MRP2 ABC transporter was assessed in vitro using MRP2 containing membrane vesicles. Transporter specific accumulation of emtricitabine in MRP2 vesicles was investigated at 2 concentrations (1 and 10 µM) and at 2 incubation times (2 and 20 minutes). Emtricitabine was incubated at 37±1°C at final concentrations of 1 and 10 µM. The amount of emtricitabine accumulated in the vesicles was determined by LC/MS method. MRP2-mediated E217βG transport in the presence or absence of 100 µM benzbromarone was carried out as a positive control for MRP2 function.

Transporter	Condition (µM/min)	Fold Accumulation
MRP2	1/2	ND
	1/20	ND
	10/2	1.06
	10/20	0.99

E217βG = estradiol-17-beta-glucuronide; FTC = emtricitabine; MRP2 = multidrug resistance-associated protein-2 (ABCC2, cMOAT); ND = not determined
Most of the samples from the 1 µM group were below the limit of quantification therefore fold accumulations could not be calculated

15. PHARMACOKINETICS: OTHER

There are no additional studies to report under this heading.